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PATENT

Docket No. 1878-4051

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

UTILITY APPLICATION AND APPLICATION FEE TRANSMITTAL (1.53(b))

ASSISTANT COMMISSIONER FOR PATENTS
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Sir:

Transmitted herewith for filing is the patent application of

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For:

INSULIN AND IGF-1 RECEPTOR AGONISTS AND ANTAGONISTS

Enclosed are:

[X] 145 page(s) of specification, 1 page(s) of Abstract, 44 page(s) of claims

[X] 199 sheets of drawing [] formal [X] informal

[X] 8 page(s) of Declaration and Power of Attorney

[X] Unsigned



- ☐ Newly Executed
☐ Copy from prior application

☐ Deletion of inventors including Signed Statement under 37 C.F.R. § 1.63(d)(2)

☒ Incorporation by Reference: The entire disclosure of the prior application is considered as being part of the disclosure of the accompanying application and is incorporated herein by reference.

☐ Microfiche Computer Program (Appendix)

☐ _____ page(s) of Sequence Listing

- ☐ computer readable disk containing Sequence Listing
☐ Statement under 37 C.F.R. § 1.821(f) that computer and paper copies of the Sequence Listing are the same

☐ Claim for Priority

☐ Certified copy of Priority Document(s)

☐ English translation documents

☐ Information Disclosure Statement

☐ Copy of _____ cited references

☐ Copy of PTO-1449 filed in parent application serial No. _____

☐ Preliminary Amendment

☒ Return receipt postcard (MPEP 503)

☐ Assignment Papers (assignment cover sheet and assignment documents)

☐ A check in the amount of \$40.00 for recording the Assignment.

☐ Assignment papers filed in parent application Serial No. _____

☐ Certification of chain of title pursuant to 37 C.F.R. § 3.73(b).

☒ This is a ☐ continuation ☐ divisional ☒ continuation-in-part (C-I-P) of prior application serial no. 09/146,127.

☐ Cancel in this application original claims _____ of the parent application before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)

☐ A preliminary Amendment is enclosed. (Claims added by this Amendment have been properly numbered consecutively beginning with the number following the highest numbered original claim in the prior application.

☒ The status of the parent application is as follows:

☐ A Petition For Extension of Time and a Fee therefor has been or is being filed in the parent application to extend the term for action in the parent application until _____.

☐ A copy of the Petition for Extension of Time in the co-pending parent application is attached.

- ☒ No Petition For Extension of Time and Fee therefor are necessary in the co-pending parent application.
- ☐ Please abandon the parent application at a time while the parent application is pending or at a time when the petition for extension of time in that application is granted and while this application is pending has been granted a filing date, so as to make this application co-pending.
- ☐ Transfer the drawing(s) from the patent application to this application.
- ☐ Amend the specification by inserting before the first line the sentence:
This is a ☐ continuation ☐ divisional ☐ continuation-in-part of co-pending application Serial No. _____
filed _____.

I. CALCULATION OF APPLICATION FEE (For Other Than A Small Entity)

	Number Filed		Number Extra	Rate	Basic Fee
Total Claims	268	-20=	248	x\$18.00	\$4464.00
Independent Claims	50	-3=	47	x\$78.00	\$3666.00
Multiple Dependent Claims					
	<input checked="" type="checkbox"/> yes		Additional Fee =	\$260.00	\$260.00
	<input type="checkbox"/> no		Add'l Fee =	NONE	

Total: \$9080.00

- ☐ A statement claiming small entity status is attached or has been filed in the above-identified parent application and its benefit under 37 C.F.R. § 1.28(a) is hereby claimed. Reduced fees under 37 C.F.R. § 1.9(F) (50% of total) paid herewith \$ _____.
- ☒ A check in the amount of \$9080.00 in payment of the application filing fees is attached.
- ☐ Charge Fee(s) to Deposit Account No. 13-4500. Order No. _____. A DUPLICATE COPY OF THIS SHEET IS ATTACHED.
- ☒ The Assistant Commissioner is hereby authorized to charge any additional fees which may be required for filing this application, or credit any overpayment to Deposit Account No. 13-4500, Order No. 1878-4051. A DUPLICATE COPY OF THIS SHEET IS ATTACHED.

Respectfully submitted,

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10/14 U.S. PTO
03/29/00

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PATENT
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Arthur J. Blume, et al. Group Art Unit: TBA
Serial No.: TBA Examiner: TBA
Filed: March 29, 2000 (Herewith)
For: INSULIN AND IGF-1 RECEPTOR AGONISTS AND ANTAGONISTS

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03/29/00

EXPRESS MAIL CERTIFICATE (37 C.F.R. §1.10)

Express Mail Label No.: EJ607481229US

Date of Deposit: March 29, 2000

I hereby certify that the following attached paper(s) and/or fee for:

1. Utility Application and Application Fee Transmittal (§ 1.53(b)); (3 Pages (In Duplicate));
2. Specification (145 Pages); Abstract (1 Page); Claims (44 Pages);
3. Informal Drawings (Figures 1-74); (199 Pages);
4. Combined Declaration and Power of Attorney (8 Pages);
5. Check (\$9080.00); and
6. Return Receipt Postcard;

is/are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. §1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

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(Typed or printed name of person
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INSULIN AND IGF-1 RECEPTOR AGONISTS AND ANTAGONISTS

This application is a continuation-in-part of U.S. Application Serial No. 09/146,127, filed September 2, 1998, which is incorporated by reference in its entirety.

5 I. FIELD OF THE INVENTION

This invention relates to the field of hormone receptor activation or inhibition. More specifically, this invention relates to the identification of molecular structures, especially peptides, which are capable of acting at either the insulin or insulin-like growth factor receptors as agonists or
10 antagonists. Also related to this invention is the field of molecular modeling whereby useful molecular structures are derived from known structures.

II. BACKGROUND OF THE INVENTION

Insulin is a potent metabolic and growth promoting hormone that acts on cells to stimulate glucose, protein, and lipid metabolism, as well as RNA
15 and DNA synthesis. A well-known effect of insulin is the regulation of the level of glucose at a whole body level. This effect by insulin occurs predominantly in liver, fat, and muscle. In liver, insulin stimulates glucose incorporation into glycogen and inhibits the production of glucose. In muscle and fat, insulin stimulates glucose uptake, storage, and metabolism.
20 Disruptions of glucose utilization are very common in the population in giving rise to diabetes.

Signal transduction in target cells is initiated by binding of insulin to a specific cell-surface receptor, the insulin receptor (IR). The binding leads to conformational changes in the extracellular domain of the receptor, which
25 are transmitted across the cell membrane and result in activation of the receptor's tyrosine kinase activity. This, in turn, leads to autophosphorylation of the insulin receptor's tyrosine kinase, and the binding of soluble effector molecules that contain SH2 domains such as

phosphoinositol-3-kinase, Ras GTPase-activating protein, and phospholipase C γ to IR (Lee and Pilch, 1994).

- Insulin-like growth factor 1 (IGF-1) is a small, single-chain protein (MW = 7,500 Da) that is involved in many aspects of tissue growth and repair, and recently has been implicated in various forms cancer including prostate, breast, colorectal, and ovarian. It is similar in size, sequence and structure to insulin, but has 100-1,000-fold lower affinity for the insulin receptor (Mynarcik *et al.*, 1997).

- Clinically, recombinant human IGF-1 has been investigated for the treatment of several diseases, including type I diabetes (Carroll *et al.*, 1997; Crowne *et al.*, 1998), amyotrophic lateral sclerosis (Lai *et al.*, 1997), and diabetic motor neuropathy (Apfel and Kessler, 1996). Other potential therapeutic applications of IGF-1 such as osteoporosis (Canalis, 1997), immune modulation (Clark, 1997) and nephrotic syndrome (Feld and Hirshberg, 1996) are being examined.

- A number of studies have analyzed the role of natural IGF-1 in various disease states. Most interestingly, several reports have shown that IGF-1 promotes the growth of normal and cancerous prostate cells both *in vitro* and *in vivo* (Angelloz-Nicoud and Binoux, 1995; Figueroa *et al.*, 1995; Torring *et al.*, 1997). Additionally, elevated serum IGF-1 levels have been connected with increased risks of prostate cancer, and may be an earlier predictor of cancer than is prostate-specific antigen (PSA) (Chan *et al.*, 1998). Recent studies have indicated a connection between IGF-1 and other cancers such as breast, colorectal, and ovarian. Serum IGF-1 levels are regulated by the presence of IGF binding proteins (IGFBP) which bind to IGF-1 and prevent its interaction with the IGF-1R (reviewed in Conover, 1996 and Rajaram *et al.*, 1997). Interestingly, PSA has been shown to be a protease that cleaves IGFBP-3, resulting in an increase of free IGF-1 in serum (Cohen *et al.*, 1992; Cohen *et al.*, 1994; Lilja, 1995). Clearly, regulation of IGF-1R activity can play an important role in several disease

states, indicating that there are potential clinical applications for both IGF-1 agonists and antagonists.

The type-1 insulin-like growth-factor receptor (IGF-1R) and insulin receptor (IR) are related members of the tyrosine-kinase receptor superfamily of growth factor receptors. Both types of receptors are composed of two α and two β subunits which form a disulfide-linked heterotetramer (β - α - α - β). They have an extracellular ligand binding domain, a single transmembrane domain, and a cytoplasmic domain displaying the tyrosine kinase activity. The extracellular domain is composed of the entire subunits and a portion of the N-terminus of the β subunits, while the intracellular portion of the β subunits contains the tyrosine kinase domain. Besides IR and IGF-1R, the other known member of the IR family is the insulin-related receptor (IRR), for which no natural ligand is known.

While similar in structure, IGF-1 and insulin receptors serve different physiological functions. The IR is primarily involved in metabolic functions whereas the IGF-1R mediates growth and differentiation. However, both insulin and IGF-1 can induce both mitogenic and metabolic effects. Whether each ligand elicits both activities via its own receptor, or whether insulin exerts its mitogenic effects through its weak affinity binding to the IGF-1 receptor, and IGF-1 its metabolic effects through the insulin receptor, remains controversial. (De Meyts, 1994).

The insulin receptor is a glycoprotein having molecular weight of 350-400 kDa (depending of the level of glycosylation). It is synthesized as a single polypeptide chain and proteolytically cleaved yielding the disulfide-linked monomer α - β insulin receptor. Two α - β monomers are linked by disulfide bonds between the α -subunits to form a dimeric form of the receptor (β - α - α - β -type configuration). The α subunit is comprised of 723 amino acids, and it can be divided into two large homologous domains, L1 (amino acids 1-155) and L2 (amino acids 313-468), separated by a cysteine rich region (amino acids 156-312) (Ward *et al.*, 1995). Many determinants

of insulin binding seem to reside in the α -subunit. A unique feature of the insulin receptor is that it is dimeric in the absence of ligand.

The sequence of IR is highly homologous to the sequence of the type-1 insulin-like growth factor receptor (IGF-1R). The homology level varies from about 40% to 70%, depending on the position within the α -subunit. The three-dimensional structures of both receptors may therefore be similar. The crystal structure of the first three domains of IGF-1R has been determined (Garrett *et al.*, 1998). The L domains consist of a single-stranded right-handed β -helix (a helical arrangement of β -strands), while the cysteine-rich region is composed of eight disulfide-bonded modules.

The β -subunit of the insulin receptor has 620 amino acid residues and three domains: extracellular, transmembrane, and cytosolic. The extracellular domain is linked by disulfide bridges to the α -subunit. The cytosolic domain includes the tyrosine kinase domain, the three-dimensional structure of which has been solved (Hubbard *et al.*, 1994).

To aid in drug discovery efforts, a soluble form of a membrane-bound receptor was constructed by replacing the transmembrane domain and the intracellular domain of IR with constant domains from immunoglobulin Fc or λ subunits (Bass *et al.*, 1996). The recombinant gene was expressed in human embryonic kidney 293 cells. The expressed protein was a fully processed heterotetramer and the ability to bind insulin was similar to that of the full-length holoreceptor.

IGF-1 and insulin competitively cross-react with IGF-1R and IR. (Schäffer, 1994). Despite 45% overall amino acid homology, insulin and IGF-1 bind only weakly to each other's receptor. The affinity of each peptide for the non-cognate receptor is about 3 orders of magnitude lower than that for the cognate receptor. (Mynarcik, *et al.*, 1997). The differences in binding affinities may be partly explained by the differences in amino acids and unique domains which contribute to unique tertiary structures of ligands. (Blakesley *et al.*, 1996).

Both insulin and IGF-1 are expressed as precursor proteins comprising, among other regions, contiguous A, B, and C peptide regions, with the C peptide being an intervening peptide connecting the A and B peptides. A mature insulin molecule is composed of the A and B chains connected by disulfide bonds, whereas the connecting C peptide has been removed during post-translational processing. IGF-1 retains its smaller C-peptide as well as a small D extension at the C-terminal end of the A chain, making the mature IGF-1 slightly larger than insulin. (Blakesley, 1996). The C region of human insulin-like growth factor (IGF-1) appears to be required for high affinity binding to the type I IGF receptor. (Pietrzkowski *et al.*, 1992). Specifically, tyrosine 31 located within this region appears to be essential for high affinity binding. Furthermore, deletion of the D domain of IGF-1 increased the affinity of the mutant IGF-1 for binding to the IR, while decreasing its affinity for the IGF-1R receptor. (Pietrzkowski *et al.*, 1992). A further structural distinction between the two hormones is that, unlike insulin, IGF-1 has very weak self-association and does not hexamerize. (De Meyts, 1994).

The α -subunits, which contain the ligand binding region of the IR and IGF-1R, demonstrate between 47-67% overall amino acid homology. Three general domains have been reported for both receptors from sequence analysis of the α subunits, L1-Cys-rich-L2. The cysteine residues in the C-rich region are highly conserved between the two receptors; however, the cysteine-rich domains have only 48% overall amino acid homology.

Despite the similarities observed between these two receptors, the role of the domains in specific ligand binding are distinct. Through chimeric receptor studies, (domain swapping of the IR and IGF-1R α -subunits), researchers have reported that the sites of interaction of the ligands with their specific receptors differ. (Blakesley, *et al.*, 1996). For example, the cysteine-rich domain of the IGF-1R (amino acids 191-290) was determined to be essential for high-affinity IGF binding, but not insulin binding by introducing this IGF-1R region into the corresponding region of the IR

(amino acids 198-300) and observing that the IR demonstrated high affinity binding of IGF-1 while maintaining high-affinity insulin binding. Conversely, when the corresponding region of the IR was introduced into the IGF-1R, the affinity for IGF-1 was not detectable while the affinity for insulin remained undisturbed.

5 A further distinction between the binding regions of the IR and IGF-1R is their differing dependence on the N-terminal and C-terminal regions. Both the N-terminal and C-terminal regions (located within the putative L1 and L2 domains) of the IR are important for high-affinity insulin binding but appear to have little effect on IGF-1 binding. Replacing residues in the N-terminus of IGF-1R (amino acids 1-62) with the corresponding residues of IR (amino acids 1-68) confers insulin-binding ability on IGF-1R. Within this region residues Phe-39, Arg-41 and Pro-42 are reported as major contributors to the interaction with insulin. (Williams *et al.*, 1995). When these residues are introduced into the equivalent site of the IGF-1R, the affinity for insulin is markedly increased, whereas, substitution of these residues by alanine in the IR results in markedly decreased insulin affinity. Similarly, the region between amino acids 704-717 of the C-terminus of IR has been shown to play a major role in insulin specificity. Substitution of these residues with alanine also disrupts insulin binding. (Mynarcik *et al.*, 1996).

Further studies of alanine scanning of the receptors suggest that insulin and IGF-1 may use some common contacts to bind to the IGF-1 receptor but that those contacts differ from those that insulin utilizes to bind to the insulin receptor. (Mynarcik *et al.*, 1997). Hence, the data in the literature has led one commentator to state that even though "the binding interfaces for insulin and IGF-1 on their respective receptors may be homologous within this interface the side chains which make actual contact and determine specificity may be quite different between the two ligand-receptor systems." (De Meyts, 1994).

The identification of molecular structures having a high degree of specificity for one or the other receptor is important to developing efficacious and safe therapeutics. For example, a molecule developed as an insulin agonist should have little or no IGF-1 activity in order to avoid the mitogenic activity of IGF-1 and a potential for facilitating neoplastic growth.

It is therefore important to determine whether insulin and IGF-1 share common three-dimensional structures but which have sufficient differences to confer selectivity for their respective receptors. Similarly, it would be desirable to identify other molecular structures which mimic the active binding regions of insulin and/or IGF-1 and which impart selective agonist or antagonist activity.

Although certain proteins are important drugs, their use as therapeutics presents several difficult problems, including the high cost of production and formulation, administration usually via injection and limited stability in the bloodstream. Therefore, replacing proteins, including insulin or IGF-1, with small molecular weight drugs has received much attention. However, none of these efforts has resulted in finding a successful drug.

Peptides mimicking functions of protein hormones have been previously reported. Yanofsky *et al.* (1996) reports the isolation of a monomer peptide antagonistic to IL-1 with nanomolar affinity for the IL-1 receptor. This effort required construction and use of many phage displayed peptide libraries and sophisticated phage panning procedures.

Wrighton *et al.* (1996) and Livnah *et al.* (1996) reported dimer peptides that bind to the erythropoietin (EPO) receptor with full agonistic activity *in vivo*. These peptides are cyclical and have intra-peptide disulfide bonds; like the IL-1 receptor antagonist, they show no significant sequence identity to the natural ligand. Importantly, X-ray crystallography revealed that it was the spontaneous formation of non-covalent peptide homodimers which enabled the dimerization two EPO receptors.

Most recently, Cwirla *et al.* (1997) reported the identification of two families of peptides that bind to the human thrombopoietin (TPO) receptor

and are competed by the binding of the natural TPO ligand. The peptide with the highest affinity, when dimerized by chemical means proved to be as potent an *in vivo* agonist as TPO, the natural ligand .

- WO 96/04557 reports the use of peptides and antibodies which bind to active sites of biological targets and which are then used in competition assays to identify small molecules which are agonist or antagonists at the biological targets.

III. SUMMARY OF THE INVENTION

- This invention relates to the identification of amino acid sequences that specifically recognize sites involved in IR and/or IGF-1R activation. Specific amino acid sequences are identified and their agonist or antagonist activity at IR or IGF-1R has been determined. Such sequences may be developed as potential therapeutics or as lead compounds to develop other more efficacious ones. In addition, these sequences may be used in high-throughput screens to identify and provide information on small molecules which bind at these sites and mimic or antagonize the functions of insulin or IGF-1. Furthermore, the peptide sequences provided by this invention can be used to design secondary peptide libraries, which can be used to identify sequence variants that increase or modulate the binding and/or activity of the original peptide at IR or IGF-1R.

- In one aspect of this invention large numbers of peptides have been screened for their IR or IGF-1R binding and activity characteristics. Analysis of their amino acid sequences has identified certain consensus sequences which may be used themselves or as core sequences in larger amino acid sequences conferring upon them agonist or antagonist activity. At least ten generic amino acid sequences have been identified which bind IR and IGF-1R with varying degrees of agonist or antagonist activity depending on the specific sequence of the various peptides identified within each motif group. Also provided are amino or carboxyl terminal extensions capable of modifying the affinity and/or pharmacological activity of the consensus sequences when part of a larger amino acid sequence.

The amino acid sequences of this invention which bind IR and/or IGF-1R include:

- a. $X_1 X_2 X_3 X_4 X_5$ wherein X_1 , X_2 , X_4 and X_5 are aromatic amino acids, and X_3 is any polar amino acid;
- 5 b. $X_6 X_7 X_8 X_9 X_{10} X_{11} X_{12} X_{13}$ wherein X_6 and X_7 are aromatic amino acids, X_8 , X_9 , X_{11} and X_{12} are any amino acid, and X_{10} and X_{13} are hydrophobic amino acids;
- c. $X_{14} X_{15} X_{16} X_{17} X_{18} X_{19} X_{20} X_{21}$ wherein X_{14} , and X_{17} are hydrophobic amino acids, X_{15} , X_{16} , X_{18} and X_{19} are any amino acid, and X_{20} and X_{21} are aromatic amino acids.
- 10 d. $X_{22} X_{23} X_{24} X_{25} X_{26} X_{27} X_{28} X_{29} X_{30} X_{31} X_{32} X_{33} X_{34} X_{35} X_{36} X_{37} X_{38} X_{39} X_{40} X_{41}$ wherein X_{22} , X_{25} , X_{28} , X_{29} , X_{30} , X_{33} , X_{34} , X_{35} , X_{36} , X_{37} , X_{38} , X_{40} , and X_{41} are any amino acid, X_{35} and X_{37} may be any amino acid for binding to IR, whereas X_{35} is preferably a hydrophobic amino acid and X_{37} is preferably glycine for binding to IGF-1R and possess agonist or antagonist activity. X_{23} and X_{26} are hydrophobic amino acids. This sequence further comprises at least two cysteine residues, preferably at X_{25} and X_{40} X_{31} and X_{32} are small amino acids.
- 15 e. $X_{42} X_{43} X_{44} X_{45} X_{46} X_{47} X_{48} X_{49} X_{50} X_{51} X_{52} X_{53} X_{54} X_{55} X_{56} X_{57} X_{58} X_{59} X_{60} X_{61}$ wherein X_{42} , X_{43} , X_{44} , X_{45} , X_{53} , X_{55} , X_{56} , X_{58} , X_{60} and X_{61} may be any amino acid, X_{43} , X_{46} , X_{49} , X_{50} , X_{54} are hydrophobic amino acids, X_{47} and X_{59} are preferably cysteines, X_{48} is a polar amino acid, and X_{51} , X_{52} and X_{57} are small amino acids.
- 20 f. $X_{62} X_{63} X_{64} X_{65} X_{66} X_{67} X_{68} X_{69} X_{70} X_{71} X_{72} X_{73} X_{74} X_{75} X_{76} X_{77} X_{78} X_{79} X_{80} X_{81}$ wherein X_{62} , X_{65} , X_{68} , X_{69} , X_{71} , X_{73} , X_{76} , X_{77} , X_{78} , X_{80} , and X_{81} may be any amino acid; X_{63} , X_{70} , X_{74} are hydrophobic amino acids; X_{64} is a polar amino acid, X_{67} and X_{75} are aromatic amino acids and X_{72} and X_{79} are preferably cysteines capable of forming a loop.
- 25 g. $H X_{82} X_{83} X_{84} X_{85} X_{86} X_{87} X_{88} X_{89} X_{90} X_{91} X_{92}$ wherein X_{82} is proline or alanine, X_{83} is a small amino acid, X_{84} is selected from leucine,
- 30

serine or threonine, X_{85} is a polar amino acid, X_{86} , X_{88} , X_{89} and X_{90} are any amino acid, and X_{87} , X_{91} and X_{92} are an aliphatic amino acid.

h. $X_{104} X_{105} X_{106} X_{107} X_{108} X_{109} X_{110} X_{111} X_{112} X_{113} X_{114}$

wherein at least one of the amino acids of X_{106} through X_{111} , and preferably two, are tryptophan separated by three amino acids, and wherein at least one of X_{104} , X_{105} and X_{106} and at least one of X_{112} , X_{113} and X_{114} are cysteine; and

i. an amino acid sequence comprising the sequence
DYKDLCSQSWGVRIGWLAGLCPKK.

j. $WX_{123} GYX_{124} WX_{125} X_{126}$ wherein X_{123} is selected from proline, glycine, serine, arginine, alanine or leucine, but more preferably proline; X_{124} is any amino acid, but preferably a charged or aromatic amino acid; X_{125} is a hydrophobic amino acid preferably leucine or phenylalanine, and most preferably leucine. X_{126} is any amino acid, but preferably a small amino acid.

In one embodiment, preferred amino acid sequences $FYX_3 WF$ ("A6" motif) and $FYX_8 X_9 L/X_{11} X_{12} L$ ("B6" motif) have been identified which competitively bind to sites on IR and IGF-1R and possess either agonist or antagonist activity. Surprisingly $FYX_3 WF$ which possesses agonist activity at IGF-1R, can possess agonist or antagonist activity at IR. Similarly, $FY X_8 X_9 L/X_{11} X_{12} L$, which is an antagonist at IGF-1R, possesses agonist activity at IR.

This invention also identifies at least two distinct binding sites on IR and IGF-1R based on the differing ability of certain of the peptides to compete with one another and insulin or IGF-1 for binding to IR and IGF-1R. Accordingly, this invention provides amino acid sequences which bind specifically to one or both sites of IR and/or IGF-1R. Furthermore, specific amino acid sequences are provided which have either agonist or antagonist characteristics based on their ability to bind to the specific sites of IR.

In another embodiment of this invention, amino acid sequences which bind to one or more sites of IR or IGF-1R may be covalently linked together

to form multivalent ligands. These multivalent ligands are capable of forming complexes with a plurality of IR or IGF-1R. Either the same or different amino acid sequences may be covalently bound together to form homo- or heterocomplexes. Dimers of the same amino acid sequence, for example, may be used to form receptor complexes bound through the same corresponding sites. Alternatively, heterodimers may be used to bind to different sites on one receptor or to cause receptor complexing through different sites.

The present invention also provides assays for identifying compounds that mimic the binding characteristics of insulin. Such compounds may act as antagonists or agonists of insulin function in cell based assays.

This invention also provides amino acid sequences such as peptides and recombinant antibody variable regions (rVab) that inhibit binding of insulin to the insulin receptor. Such amino acid sequences and rVabs are used in the assays of the invention to identify compounds that mimic insulin.

This invention also provides kits for identifying compounds that bind to the insulin receptor. The invention further provides therapeutic compounds that bind the insulin receptor.

In another embodiment, this invention provides assays for identifying compounds which mimic the binding characteristics of IGF-1. Such compounds act as antagonists or agonists of IGF-1 hormone function in cell based assays.

The invention also provides amino acid sequences such as peptides and rVabs which inhibit binding of IGF-1 to IGF-1R. Such amino acid sequences and rVabs are used in the assays of the invention to identify compounds which mimic IGF-1.

Another embodiment of this invention is the nucleic acid sequences encoding the amino acid sequences of the invention. Also within the scope of this invention are vectors containing the nucleic acids and host cells which express the genes encoding the amino acid sequences which bind at IR or IGF-1R and possess agonist or antagonist activity.

It is an object of this invention to provide amino acid sequences which bind to active sites of IR and/or IGF-1R and to identify structural criteria for conferring agonist or antagonist activity at IR and/or IGF-1R.

5 It is a further object of this invention to provide specific amino acid sequences which possess agonist, partial agonist or antagonist activity at either IR or IGF-1R. Such amino acid sequences are potentially useful as therapeutics themselves or may be used to identify other molecules, especially small organic molecules, which possess agonist or antagonist activity at IR or IGF-1R.

10 Another object of this invention is to provide structural information derived from the amino acid sequences of this invention which may be used to construct other molecules possessing the desired activity at the relevant IR of IGF-1R binding site.

IV. BRIEF DESCRIPTION OF THE DRAWINGS

15 Figures 1A-10G. Amino acid sequences comprising the motif of Formulas 1 through 10. Sequences were identified by panning peptide libraries against IGF-1R and/or IR. The amino acids are represented by their one-letter abbreviation. The ratios over background are determined by dividing the signal at 405 nm (E-Tag, IGF-1R, or IR) by the signal at 405 nm for non-fat milk. The IGF-1R/IR Ratio Comparison is determined by dividing the ratio of IGF-1R by the ratio of IR. The IR/IGF-1R Ratio Comparison is determined by dividing the ratio of IR by the ratio of IGF-1R.

25 The design of each library is shown in the first line in bold. In the design, symbol 'X' indicates a random position, an underlined amino acid indicates a doped position at the nucleotide level, and other positions are held constant. Additional abbreviations in the B6H library are: 'O' indicates an NGY codon where Y is C or T; 'J' indicates an RHR codon where R is A or G, and H is A, C, or T; and 'U' indicates an VVY codon where V is A, C, or G, and Y is C or T. The 'h' in the 20E2 libraries indicates an N_hIN codon.

30 Symbols in the listed sequences are: Q - TAG Stop; # -TAA Stop; * - TGA Stop; and ? - Unknown Amino Acid. It is believed that a W replaces

the TGA Stop Codon when expressed. Except for the 20C, A6L, and B6L libraries, all libraries are designed with the short FLAG Epitope DYKD (Hopp *et al.*, 1988) at the N-terminus of the listed sequence and AAAGAP at the C-terminus. The 20C, A6L, and B6L libraries have the full length FLAG

5 epitope DYKDDDDDK.

Figure 1A: Formula 1 motif peptide sequences obtained from a random 40mer library panned against IR.

Figure 1B: Formula 1 motif peptide sequence obtained from a random 40mer library panned against IGF-1R.

10 Figure 1C: Formula 1 motif peptide sequences obtained from a random 20mer library panned against IR.

Figure 1D: Formula 1 motif peptide sequences obtained from a random 20mer library panned against IGF-1R.

15 Figure 1E: Formula 1 motif peptide sequences obtained from a 21mer library constructed to contain X_{1-10} NFYDWFVX₁₈₋₂₁ (also referred to as "A6S") panned against IR.

Figure 1F: Formula 1 motif peptide sequences obtained from a 21mer library constructed to contain X_{1-10} NFYDWFVX₁₈₋₂₁ (also referred to as "A6S") panned against IGF-1R.

20 Figure 1G: Formula 1 motif peptide sequences obtained from a library constructed to contain variations outside the consensus core of the A6 peptide as indicated (referred to as "A6L") panned against IR.

Figure 1H: Formula 1 motif peptide sequences obtained from a library constructed to contain variations outside the consensus core of the A6 peptide as indicated (referred to as "A6L") panned against IGF-1R.

25 Figure 1I: Formula 1 motif peptide sequences obtained from a library constructed to contain variations in the consensus core of the E4D peptide (as indicated) panned against IR.

30 Figure 1J: Formula 1 motif peptide sequences obtained from a library constructed to contain variations in the consensus core of the E4D peptide (as indicated) panned against IGF-1R.

Figure 1K: Formula 1 motif peptide sequences obtained from a library constructed using the sequence X_{1-6} FHENFYDWVFRQVSX X_{21-26} (H2C-A) panned against IR.

Figure 1L: Formula 1 motif peptide sequences obtained from a library constructed using the sequence X_{1-6} FHENFYDWVFRQVSX X_{21-26} (H2C-A) panned against IGF-1R.

Figure 1M: Formula 1 motif peptide sequences obtained from a library constructed using the sequence X_{1-6} FHXXFYXWFX X_{16-21} (H2C-B) and panned against IR.

Figure 1N: Formula 1 motif peptide sequences obtained from a library constructed using the sequence X_{1-6} FHXXFYXWFX X_{16-21} (H2C-B) and panned against IGF-1R.

Figure 1O: Formula 1 motif peptide sequences obtained from other libraries panned against IR.

Figure 2A: Formula 2 motif peptide sequence identified from a random 40mer library panned against IR.

Figure 2B: Formula 2 motif peptide sequences identified from a random 40mer library panned against IGF-1R.

Figure 2C: Formula 2 motif peptide sequences identified from a random 20mer library panned against IR.

Figure 2D: Formula 2 motif peptide sequences identified from a random 20mer library panned against IGF-1R.

Figure 2E: Formula 2 motif peptide sequences identified from a $X_{1-4}CX_{6-20}$ library panned against IGF-1R.

Figure 2F: Formula 2 motif peptide sequences identified from a library constructed to contain variations outside the consensus core of the B6 peptide as indicated (referred to as "B6L") and panned against IR.

Figure 2G: Formula 2 motif peptide sequences identified from a library constructed to contain variations outside the consensus core of the B6 peptide as indicated (referred to as "B6L") and panned against IGF-1R.

Figure 2H: Formula 2 motif peptide sequences identified from a library constructed to contain a helix-turn-helix based on the B6 peptide as indicated (referred to as "B6H") and panned against IR.

5 Figure 2I: Formula 2 motif peptide sequences identified from a library constructed to contain a helix-turn-helix based on the B6 peptide as indicated (referred to as "B6H") and panned against IGF-1R.

Figure 2J: Formula 2 motif peptide sequences identified from a library constructed to contain variations in the consensus core of B6 peptide as indicated (referred to as "B6C") and panned against IR.

10 Figure 2K: Formula 2 motif peptide sequences identified from a library constructed to contain variations in the consensus core of B6 peptide as indicated (referred to as "B6C") and panned against IGF-1R.

Figure 2L: Formula 2 motif peptide sequences identified from a library constructed using the sequence $X_{1-6}\text{FYDAIDQLVX}_{16-21}$ (20E2-A)
15 panned against IR.

Figure 2M: Formula 2 motif peptide sequences identified from a library constructed using the sequence $X_{1-6}\text{FYDAIDQLVX}_{16-21}$ (20E2-A) panned against IGF-1R.

Figure 2N: Formula 2 motif peptide sequences identified from a
20 library constructed using the sequence $X_{1-6}\text{FYXXhXXhhX}_{16-21}$ (20E2-B) panned against IR.

Figure 2O: Formula 2 motif peptide sequences identified from a library constructed using the sequence $X_{1-6}\text{FYXXhXXhhX}_{16-21}$ (20E2-B) panned against IGF-1R.

25 Figure 2P: Formula 2 motif peptide sequences identified from a library constructed using the sequence $X_{1-6}\text{FYRYFXXLLX}_{16-21}$ (NNRP) panned against IR.

Figure 3A: Formula 3 motif peptide sequences identified from a random 20mer library panned against IGF-1R.

30 Figure 3B: Formula 3 motif peptide sequences identified from a $X_{1-4}\text{CX}_{6-20}$ library panned against IGF-1R.

Figure 3C: Formula 3 motif peptide sequences identified from a library constructed using the sequence X₃LXXLXXYFX₁₂₋₁₇ (reverse B6; rB6) panned against IR.

5 Figure 3D: Formula 3 motif peptide sequences identified from a library constructed using the sequence X₃LXXLXXYFX₁₂₋₁₇ (reverse B6; rB6) panned against IGF-1R.

Figure 4A: Formula 4 motif peptide sequences identified from a random 20mer library panned against IR.

10 Figure 4B: Formula 4 motif peptide sequences identified from a library constructed to contain variations in the F8 peptide as indicated (15% dope; referred to as "F815") panned against IR.

Figure 4C: Formula 4 motif peptide sequences identified from a library constructed to contain variations in the F8 peptide as indicated (15% dope; referred to as "F815") panned against IGF-1R.

15 Figure 4D: Formula 4 motif peptide sequences identified from a library constructed to contain variations in the F8 peptide as indicated (20% dope; referred to as "F820") panned against IR.

Figure 4E: Formula 4 motif peptide sequences identified from other libraries panned against IR.

20 Figure 5: Formula 5 motif peptide sequences identified from a library constructed to contain variations in the F8 peptide as indicated (15% dope; referred to as "F815") panned against IGF-1R.

Figure 6A: Formula 6 motif peptide sequences identified from a random 20mer library and panned against IR.

25 Figure 6B: Formula 6 motif peptide sequences identified from a library constructed to contain variations in the D8 peptide as indicated (15% dope; referred to as "D815") panned against IR.

Figure 6C: Formula 6 motif peptide sequences identified from a library constructed to contain variations in the D8 peptide as indicated (20% dope; referred to as "D820") panned against IR.

30

Figure 6D: Formula 6 motif peptide sequences identified from a library constructed to contain variations in the D8 peptide as indicated (20% dope; referred to as "D820") panned against IGF-1R.

5 Figure 6E: Formula 6 motif peptide sequences identified from other libraries panned against IR.

Figure 7: Formula 7 motif peptide sequences.

Figure 8: Formula 8 motif peptide sequences identified from a commercial phage display peptide library and synthetic sequences. Small letters denote D-amino acids. Unnatural amino acids are denoted with a 3-letter abbreviation in certain sequences. K_d values greater than 2×10^{-5} are approximate.

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Figure 9A: Formula 9 motif peptide sequences identified from a library constructed to contain variations in the H5 peptide as indicated (referred to as "H5") panned against IGF-1R.

15 Figure 9B: Formula 9 motif peptide sequences identified from a library constructed to contain variations in the JBA5 peptide as indicated (referred to as "JBA5") panned against IGF-1R.

Figure 9C: Formula 9 motif peptide sequences identified from a library constructed to contain variations in the JBA5 peptide as indicated (referred to as "JBA5") panned against IR.

20

Figure 10A: Formula 10 motif peptide sequences identified from random 20mer libraries panned against IGF-1R.

Figure 10B: Formula 10 motif peptide sequences identified from random 20mer libraries panned against IR.

25 Figure 10C: Miscellaneous peptide sequences identified from a random 20mer library panned against IR.

Figure 10D: Miscellaneous peptide sequences identified from a random 40mer library panned against IR.

30 Figure 10E: Miscellaneous peptide sequences identified from a random 20mer library panned against IGF-1R.

Figure 10F: Miscellaneous peptide sequences identified from a X₁-₄CX₆₋₂₀ and panned against IGF-1R.

Figure 10G: Miscellaneous peptide sequences identified from a library constructed to contain variations of the F8 peptide as indicated (F815) panned against IGF-1R.

Figure 10H: Miscellaneous peptide sequences identified from a library constructed to contain variations in the F8A11 peptide as indicated (referred to as "NNKH") panned against IR.

Figure 10I: Miscellaneous peptide sequences identified from a library constructed to contain variations in the F8A11 peptide as indicated (referred to as "NNKH") panned against IGF-1R.

Figure 11A: Summary of specific representative amino acid sequences from Formulas 1 through 11.

Figure 11B: Summary of specific representative amino acid sequences from Formulas 1 through 11.

Figure 12: Illustration of helix wheels applied to Formula 2 and 3 motifs.

Figure 13: Illustration of 2 binding site domains on IR based on competition data.

Figure 14: Dissociation of 20E2 peptide from IGF-1R in the presence of buffer (filled circle), 30 μ M IGF-1 (open circle), 100 μ M H2C (filled square), 100 μ M 20E2 (filled triangle), 100 μ M D8 (B12; open square), 100 μ M C1 (filled, inverted triangle) and 100 μ M RPG (filled diamond).

Figure 15: Schematic illustration of potential binding schemes to the multiple binding sites on IR.

Figure 16: Schematic diagram of the phage-displayed peptide library. The peptide is displayed as a protein fusion to the N-terminus of gene III encoding the minor coat protein of the phage.

Figure 17: BIAcore analysis of competition binding between IR and MBP fusion H2C-9-H2C, H2C and H2C-3-H2C.

Figure 18: Sequence alignments of Class I and Class II peptides. The Class I peptides have been shown to be IGF-1R antagonists, while the Class II peptides are IGF-1R agonists.

Figure 19: DNA sequences of the frameshifted clones.

- 5 Figures 20A and 20B: Results of the phage ELISA for binding to IGF-1R. Wells were coated with 100 ng/well IGF-1R and blocked. Competitor, the IGF-1 native ligand, was present prior (1 h) and during the phage incubation (1 h). Phage were detected with HRP-anti M13 phage antibody and reported as OD₄₀₅ as described. Total Binding is shown in Figure 20A and Percent Inhibition is shown in Figure 20B.
- 10

Figure 21: Sequences of the designed IGF-1R-specific synthetic peptides.

Figure 22: Assay results showing that Motif 2 peptides (5.1 and 5.2) antagonize the effects of IGF-1 on IGF-1R⁺ cells.

- 15 Figure 23: Assay results showing that Motif 1 peptides (5.3 and 5.4) stimulate growth of IGF-1R⁺ cells. Cells expressing human IGF-1R (30,000 cells per well) were incubated with the 5.4 peptide for 42 h at 37°C. Experiments were done in triplicate. Background signal A₄₅₀=0.15. Proliferation was measured using WST-1 reagent (Boehringer Mannheim Biochemicals/Roche Molecular Biochemicals, Indianapolis, IN).
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Figures 24A and 24B: Demonstration of binding of peptide 5.1 to IGF-1R using BIAcore. Figure 24A. Binding as a function of the peptide concentration. Figure 24B: Inhibition of IGF-1 binding by peptide 5.1. RU – refractive units.

- 25 Figures 25A and 25B: Design of the secondary phage library A6L based on the Class II peptide sequences. Figure 25A: Design of the sequence of the gene. Underlined residues indicate positions mutated to optimize the codons for expression in *E. coli*. Figure 25B: Synthetic oligonucleotide for the A6L secondary library. Underlined residues were doped in the chemical DNA synthesis. Definitions of mixes (all mixes are equimolar) are as follows: N = A, C, G, or T; K = G or T. Nucleosides were
- 30

premixed in the bottle (and not line mixed) to improve the accuracy of nucleoside mixes. The sequence of the FLAG epitope is shown in bold.

Figures 26A and 26B: Design of the secondary phage library A6S based on the Motif 1 peptide sequences. Figure 26A: Sequence design for the A6S secondary phage library. Figure 26B: Synthetic oligonucleotide for the A6L secondary library. Definitions of mixes (all mixes are equimolar) are as follows: N = A, C, G, or T; K = G or T. Nucleosides were premixed in the bottle (and not line mixed) to improve the accuracy of nucleoside mixes. The sequence of the FLAG epitope is shown in bold.

Figure 27: Sequences of the five H5-like peptides that show agonistic activity toward IGF-1R. The C-terminal lysine contains a biotin moiety linked to the amino group of the side chain.

Figure 28: Listing of amino acid sequences obtained from panning with the A6S library.

Figure 29: Listing of amino acid sequences obtained from panning with the H5 secondary phage library.

Figure 30: Schematic of the genomic rVab library.

Figure 31: Listing of the V_H, kappa and lambda genes used to assemble the rVab antibody library for IGF-1R binders.

Figure 32: Schematic of the assembly of the single-chain IGF-I and insulin antibody libraries from restriction fragments.

Figure 33: Sequences of the restriction fragments used to assemble the rVab libraries.

Figure 34: Nucleotide sequence of the gene encoding the 43G7 rVab specific for IGF-1R. The predicted amino acid sequence of the rVab is shown below the nucleic acid sequence.

Figure 35: Nucleotide sequence of the gene encoding the 1G2P rVab specific for IGF-1R. The predicted amino acid sequence of the rVab is shown below the nucleic acid sequence.

Figure 36: Nucleotide sequence of gene encoding the 39F7 rVab specific for IGF-1R. The predicted amino acid sequence of the rVab is shown below the nucleic acid sequence.

5 Figure 37: Nucleotide sequence of gene encoding the M100 rVab specific for IGF-1R. The predicted protein sequence of the rVab is shown below the nucleic acid sequence.

Figure 38: Nucleotide sequence of gene encoding the 46A7 rVab specific for IGF-1R. The predicted protein sequence of the rVab is shown below the nucleic acid sequence.

10 Figure 39: Nucleotide sequence of gene encoding the 49E8 rVab specific for IGF-1R. The predicted protein sequence of the rVab is shown below the nucleic acid sequence.

Figure 40: Assay results demonstrating the binding of soluble forms of three rVabs to IGF-1R.

15 Figure 41: Assay results showing that the 43G7 rVab stimulates growth of IGF-1R⁺ cells.

Figure 42: Assay results showing that the stimulation by rVab 43G7 is antagonized by the 1G2P, 49E8, and 46A7 rVabs. The assay was done on IGF-1R⁺ cells.

20 Figure 43: Eu-based fluorescence assay results showing that the binding of peptide 5.1 to IGF-1R can be competed by the IGF-1 ligand.

Figure 44: Results of the time-resolved fluorescence assay showing that the binding of 43G7 rVab to IGF-1R is effectively competed by IGF-1.

25 Figure 45: Eu-based fluorescence assay showing that the binding of the B6 peptide to IGF-1R is effectively competed by the 43G7 rVab.

Figures 46A-46D: Results of the Eu-based fluorescence assay showing that the binding of the europium-labeled 43G7 rVab to IGF-1R is effectively competed by selected scAbs specific for IGF-1R.

30 Figure 47: Biopanning results and sequence alignments of Group 1 of IR-binding peptides. The number of sequences found is indicated on the right side of the figure together with data on the phage binding to either IR or

IGF-1R receptor. Absorbance signals are indicated by: +++, >30X over background; ++, 15-30X; +, 5-15X; +, 2-5X; and 0, <2X.

Figure 48: Biopanning results and sequence alignments of Groups 2 through 7 of IR-binding peptides. The number of sequences found is indicated on the right side of the figure together with data on the phage binding to either IR or IGF-1R receptor.

Figure 49A-49D: Dose response curve of D118 peptide (Formula 2 motif) stimulated increase of ^3H -glucose into mouse adipocytes.

Figures 50A-50D: Titration of the synthetic peptides C1 (Figures 50A, 50C) or B6 (Figures 50B, 50D) against constant concentration of phage bound to IR (Figures 50A, 50B) or IGF-1R (Figures 50C, 50D). Phage are represented by: open circle – 20D3; open square – 20A4; open triangle – 20E2; open diamond – F2; filled circle – F8; and filled square – D8.

Figure 51A-51D: Titration of the IGF-1R synthetic peptides against constant concentration of phage. Symbols for the peptides are: open circles – H2; filled circles – H2C; open square – C1; filled square – C1C; open triangle – D2C; filled triangle – E4; open diamond – A6; and filled diamond p53.

Figure 52A-52D: Hill plot analysis of phage clones. The detailed data are provided in Table 7. Symbols are the same as in Figure 51.

Figure 53: Competition between the insulin and the IR-binding phage. The results for seven different groups (categories) of phage binders are shown.

Figure 54: Titration of the synthetic peptide 20A4 against constant concentration of phage. Phage binding to IR are represented by: open circle – 20D3; filled circle B8; open square – 20A4; filled square – D8; open up triangle – 20E2; open down triangle – D10; filled down triangle – A2; open diamond – F2; filled diamond – E8; and cross-filled circle – F8.

Figure 55: A schematic drawing for the construction of protein fusions of the maltose binding protein and peptides from phage libraries.

Figure 56A-56C: Insulin Receptor Competition ELISA using MBP-Peptide Fusion Proteins. Figure 54A. Competition with fusion proteins containing cysteine residues. The hatched bars indicate value is $\leq 54\%$ control value. Figure 54B. Competition with fusion proteins containing the consensus sequence. The notation, c-c, indicates phage displayed peptides with cysteine residues. Figure 54C. Competition with fusion protein containing a control peptide.

Figure 57: Nucleotide and predicted amino acid sequence of the gene encoding the 6f6 rVab that binds to IR.

Figure 58: Nucleotide and predicted amino acid sequence of the gene encoding the 14c8 rVab that binds to IR.

Figure 59: Comparison of the VH CDR3 sequences of different rVabs that bind to IR, and competitions of these rVabs and insulin for binding to IR.

Figure 60: Biological response of insulin, rVab 12h10, and rVab 13h9 in 32D cells expressing or not expressing IR.

Figure 61: Competition of rVab 6f6 and insulin for binding to IR.

Figure 62: Competition of rVab 6f6 and IGF-1 for binding to IR.

Figure 63: Competition of synthetic peptides and soluble rVab antibodies for binding of biotinylated peptides to insulin receptor. Synthetic peptides or soluble rVab at indicated concentrations were incubated with biotinylated peptides overnight using the heterogeneous TRFA.

Figure 64: Binding of C1 to IR and IGF-1R.

Figure 65: Competition of peptides for binding to IR.

Figure 66: H2C competition for b-peptide binding to IR. Biotinylated peptides at indicated concentrations were competed by increasing concentrations of H2C for binding to IR using the heterogeneous TRFA.

Figure 67: C1C competition for b-C1 binding to IR. Biotinylated C1 peptide at $0.3\ \mu\text{M}$ was competed by increasing concentrations of C1C for binding to IR using the heterogeneous TRFA.

Figure 68: Competition of peptides for binding of rVab 12H10 to insulin receptor. Synthetic peptides at indicated concentration were incubated with rVab 12H10 overnight using the heterogeneous TRFA.

Figure 69: Competition of MBP-peptide fusion proteins to rVab 12H10 binding to insulin receptor. Four MBP-peptides fusion proteins at indicated concentrations were incubated with rVab 12H10 overnight using the heterogeneous TRFA.

Figures 70A-70N: Peptide binding displacement curves showing the displacement of 125 I-insulin or 125 IGF-1 from HIR or HIGF-1R in the presence of various peptides.

Figures 71A-71Z; 71A2-71Z2; 71A3-71B3: Concentration dependent modulation of 3 H-glucose into adipocytes by various peptides. Formula 1 motif peptide responses are shown in Figures 71A-71V; 71A2-71J2; Formula 9 motif peptide response is shown in Figures 71W-71Z; Formula 2 motif peptide response is shown in Figures 71K2-71L2; Miscellaneous peptide motif 10 peptide responses are shown in Figures 71M2-71P2; Formula 6 motif peptide response is shown in Figure 71Q2-Figure 71R2; and Formula 4 motif peptide response is shown in Figure 71S2-Figure 71W2. Formula 1 and Formula 2 motif peptide response is shown in Figure 71X2-Figure 71A3. Fusion peptide S291 response is shown in Figure 71B3.

Figures 72A and 72B: Competition of Site 1(Figure 72B) and Site 2 (Figure 72A) phage displayed peptides with recombinant cleaved dipeptides.

Figure 73: Competition of IGF-1R, peptide H2C (D117), peptide C1 (D112), and peptide RP6 (20C-3-G3-IGFR) in a homogeneous fluorescent-resonance energy transfer assay based on the binding of IGF-1R to peptide 20E2 (D118).

Figure 74: Stimulation of IR autophosphorylation *in vivo* by MBP-fusion peptides.

V. DETAILED DESCRIPTION OF THE INVENTION

This invention relates to amino acid sequences comprising motifs which bind to the IGF-1 receptor (IGF-1R) and/or the insulin receptor (IR).

In addition to binding to IR and IGF-1R, the amino acid sequences also possess either agonist, partial agonist or antagonist activity at one or both of these receptors. Based on the differing regions of IR and IGF-1R which are reported to be important for binding and activity, this invention surprisingly

5 provides amino acid sequences which define common binding motifs on IR and IGF-1R which are capable of conferring agonist and/or antagonist activity at these receptors. In addition, this invention identifies multiple binding sites (Sites 1 and 2) on IR and IGF-1R which appear to be allosterically coupled.

10 Although capable of binding to IR and/or IGF-1R at sites which participate in conferring agonist or antagonist activity, the amino acid sequences are neither based on insulin or IGF-1 native sequences, nor do they reflect an obvious homology to any such sequence.

The amino acid sequences of the invention may be peptides,

15 polypeptides, or proteins. These terms as used herein should not be considered limiting with respect to the size of the various amino acid sequences referred to herein and which are encompassed within this invention. Thus, any amino acid sequence comprising at least one of the IR or IGF-1R binding motifs disclosed herein, and which binds to one of the

20 receptors is within the scope of this invention. In preferred embodiments, the amino acid sequences confer insulin or IGF agonist or antagonist activity. The amino acid sequences of the invention are typically artificial, i.e. non-naturally occurring peptides or polypeptides. Amino acid sequences useful in the invention may be obtained through various means such as

25 chemical synthesis, phage display, cleavage of proteins or polypeptides into fragments, or by any means which amino acid sequences of sufficient length to possess binding ability may be made or obtained.

The amino acid sequences provided by this invention should have an affinity for IR or IGF-1R sufficient to provide adequate binding for the

30 intended purpose. Thus, for use as a therapeutic, the peptide, polypeptide or protein provided by this invention should have an affinity (K_d) of between

about 10^{-7} to about 10^{-15} M. More preferably the affinity is 10^{-8} to about 10^{-12} M. Most preferably, the affinity is 10^{-9} to about 10^{-11} M. For use as a reagent in a competitive binding assay to identify other ligands, the amino acid sequence preferably has affinity for the receptor of between about 10^{-5} to about 10^{-12} M.

A further consideration in identifying peptides provided by this invention for use as therapeutics is the relative activity at either IR or IGF-IR. Thus, a peptide which has efficacy at IR and clinically insignificant activity of IGF-IR may be a useful therapeutic even though such a peptide may bind IGF-IR with relatively high affinity.

At least ten different binding motifs have been identified which bind to active sites on IR; at least four of these also bind to IGF-1R. The binding motifs are defined based on the analysis of several different amino acid sequences and analyzing the frequency that particular amino acids or types of amino acids occur at a particular position of the amino acid sequence.

For the purposes of this invention, the amino acids are grouped as follows: amino acids possessing alcohol groups are serine (S) and threonine (T). Aliphatic amino acids are isoleucine (I), leucine (L), valine (V), and methionine (M). Aromatic amino acids are phenylalanine (F), histidine (H), tryptophan (W), and tyrosine (Y). Hydrophobic amino acids are alanine (A), cysteine (C), phenylalanine (F), glycine (G), histidine (H), isoleucine (I), lysine (L), methionine (M), arginine (R), threonine (T), valine (V), tryptophan (W), and tyrosine (Y). Negative amino acids are aspartic acid (D) and glutamic acid (E). The following amino acids are polar amino acids: cysteine (C), aspartic acid (D), glutamic acid (E), histidine (H), lysine (K), asparagine (N), glutamine (Q), arginine (R), serine (S), and threonine (T). Positive amino acids are histidine (H), lysine (K), and arginine (R). Small amino acids are alanine (A), cysteine (C), aspartic acid (D), glycine (G), asparagine (N), proline (P), serine (S), threonine (T), and valine (V). Very small amino acids are alanine (A), glycine (G) and serine (S). Amino acids likely to be involved in a turn formation are alanine (A), cysteine (C),

aspartic acid (D), glutamic acid (E), glycine (G), histidine (H), lysine (K), asparagine (N), glutamine (Q), arginine (R), serine (S), proline (P), and threonine (T).

- 5 The amino acids within each of these defined groups may be substituted for each other in the motifs described below, subject to the specific preferences stated herein. In addition, synthetic or non-naturally occurring amino acids may also be used in accordance with this invention.

- Also included within the scope of this invention are amino acid sequences containing substitutions, additions, or deletions based on the teachings disclosed herein and which bind to IR or IGF-1R with the same or altered affinity. For example, amino acid residues located at the carboxy and amino terminal regions of the consensus motifs described below, which amino acid residues are not associated with a strong preference for a particular amino acid, may optionally be deleted providing for truncated sequences. Certain amino acids such as lysine which promote the stability of the amino acids sequences may be deleted depending on the use of the sequence, as for example, expression of the sequence as part of a larger sequence which is soluble, or linked to a solid support.
- 15

- Peptides that bind to IGF-1R, and methods and kits for identifying such peptides, have been disclosed by Beasley et al., U.S. Application Serial No. 09/146,127, filed September 2, 1998, which is incorporated by reference in its entirety.
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A. Consensus Motifs

- The following motifs have been identified as conferring binding activity to IR and/or IGF-1R:
- 25

1. $X_1X_2X_3X_4X_5$ (Formula 1, the A6 motif) wherein X_1 , X_2 , X_4 and X_5 are aromatic amino acids, preferably, phenylalanine or tyrosine. Most preferably, X_1 and X_5 are phenylalanine and X_2 is tyrosine. X_3 may be any small polar amino acid, but is preferably selected from aspartic acid, glutamic acid, glycine, or serine, and is most preferably aspartic acid or
- 30

glutamic acid. X_4 is most preferably tryptophan, tyrosine, or phenylalanine and most preferably tryptophan. Particularly preferred embodiments of the A6 motif are FYDWF and FYEWF. The A6 motif possesses agonist activity at IGF-1R, but agonist or antagonist activity at IR depending on the identity of amino acids flanking A6. See Figure 11A. Two amino acid sequences comprising the A6 motif possess agonist activity at IR are FHENFYDWFVRQVSKK (D117; H2C) and GRVDWLQRNANFYDWFVAELG-NH₂ (S175). Nonlimiting examples of Formula 1 amino acid sequences are shown in Figures 1A-1O.

- 10 2. $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$ (Formula 2, the B6 motif) wherein X_6 and X_7 are aromatic amino acids, preferably, phenylalanine or tyrosine. Most preferably, X_6 is phenylalanine and X_7 is tyrosine. X_8 , X_9 , X_{11} and X_{12} may be any amino acid. X_{10} and X_{13} are hydrophobic amino acids, preferably leucine, isoleucine, phenylalanine, tryptophan or methionine, but
15 more preferably leucine or isoleucine. X_{10} is most preferably isoleucine for binding to IR and leucine for binding to IGF-1R. X_{13} is most preferably leucine. Amino acid sequences of Formula 2 may function as an antagonist at the IGF-1R, or as an agonist at the IR. Preferred consensus sequences of the Formula 2 motif are $FYX_8X_9LX_{11}X_{12}L$, $FYX_8X_9IX_{11}X_{12}L$, FYX_8
20 $AIX_{11}X_{12}L$, and $FYX_8YFX_{11}X_{12}L$.

Another Formula 2 motif for use with this invention comprises $FYX_8YFX_{11}X_{12}L$ and is shown as Formula 2A ("NNRP") below:

$X_{115}X_{116}X_{117}X_{118}FYX_8YFX_{11}X_{12}LX_{119}X_{120}X_{121}X_{122}$,

wherein X_{115} - X_{118} and X_{119} - X_{122} may be any amino acid which allows for

- 25 binding to IR or IGF-1R. X_{115} is preferably selected from the group consisting of tryptophan, glycine, aspartic acid, glutamic acid and arginine. Aspartic acid, glutamic acid, glycine, and arginine are more preferred. Tryptophan is most preferred. The preference for tryptophan is based on its presence in clones at a frequency three to five fold higher than that
30 expected over chance for a random substitution, whereas aspartic acid,

glutamic acid and arginine are present about two fold over the frequency expected for random substitution.

- 5 X_{116} preferably is an amino acid selected from the group consisting of aspartic acid, histidine, glycine, and asparagine. X_{117} and X_{118} are preferably glycine, aspartic acid, glutamic acid, asparagine or alanine. More preferably X_{117} is glycine, aspartic acid, glutamic acid and asparagine whereas X_{118} is more preferably glycine, aspartic acid, glutamic acid or alanine.

- 10 X_8 when present in the Formula 2A motif is preferably arginine, glycine, glutamic acid, or serine.

X_{11} when present in the Formula 2A motif is preferably glutamic acid, asparagine, glutamine, or tryptophan, but most preferably glutamic acid.

X_{12} when present in the Formula 2A motif is preferably aspartic acid, glutamic acid, glycine, lysine or glutamine, but most preferably aspartic acid.

- 15 X_{119} is preferably glutamic acid, glycine, glutamine, aspartic acid or alanine, but most preferably glutamic acid.

X_{120} is preferably glutamic acid, aspartic acid, glycine or glutamine, but most preferably glutamic acid.

- 20 X_{121} is preferably tryptophan, tyrosine, glutamic acid, phenylalanine, histidine, or aspartic acid, but most preferably tryptophan or tyrosine.

X_{122} is preferably glutamic acid, aspartic acid or glycine; but most preferably glutamic acid.

- 25 Preferred amino acid residue are identified based on their frequency in clones over two fold over that expected for a random event, whereas the more preferred sequences occur about 3-5 times as frequently as expected.

Nonlimiting examples of amino acid sequences having the Formula 2 and 2A motifs are described in Figures 2A-2P.

3. $X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}$ (Formula 3, reverse B6, revB6), wherein X_{14} and X_{17} are hydrophobic amino acids; X_{14} , X_{17} are preferably leucine, isoleucine, and valine, but most preferably leucine; X_{15} , X_{16} , X_{18} and X_{19} may be any amino acid; X_{20} is an aromatic amino acid, preferably
- 30

tyrosine or histidine, but most preferably tyrosine; and X_{21} is an aromatic amino acid, but preferably phenylalanine or tyrosine, and most preferably phenylalanine. For use as an IGF-1R binding ligand, an aromatic amino acid is strongly preferred at X_{18} . See Figures 3A-3D for nonlimiting

5 examples of Formula 3 amino acid sequences.

4. $X_{22}X_{23}X_{24}X_{25}X_{26}X_{27}X_{28}X_{29}X_{30}X_{31}X_{32}X_{33}X_{34}X_{35}X_{36}X_{37}X_{38}X_{39}X_{40}X_{41}$
(Formula 4, "F8") wherein X_{22} , X_{25} , X_{26} , X_{28} , X_{29} , X_{30} , X_{33} , X_{34} , X_{35} , X_{36} , X_{37} ,
 X_{38} , X_{40} , and X_{41} are any amino acid. X_{35} and X_{37} may be any amino acid
when the F8 motif is used as an IR binding ligand or as a component of an

- 10 IR binding ligand, however for use as an IGF-1R binding ligand, glycine is strongly preferred at X_{37} and a hydrophobic amino acid, particularly, leucine, is preferred at X_{35} . X_{23} is a hydrophobic amino acid. Methionine, valine, leucine or isoleucine are preferred amino acids for X_{23} , however, leucine which is most preferred for preparation of an IGF-1R binding ligand is
- 15 especially preferred for preparation of an IR binding ligand. At least one cysteine is located at X_{24} through X_{27} , and one at X_{39} or X_{40} . Together the cysteines are capable of forming a cysteine cross-link to create a looped amino acid sequence. In addition, although a spacing of 14 amino acids in between the two cysteine residues is preferred, other spacings may also be
- 20 used provided binding to IGF-1R or IR is maintained. Accordingly, other amino acids may be substituted for the cysteines at positions X_{24} and X_{39} if the cysteines occupy other positions. In one embodiment, for example, the cysteine at position X_{24} may occur at position X_{27} which will produce a smaller loop provided that the cysteine is maintained at position X_{39} . These
- 25 smaller looped peptides are described herein as Formula 5, infra. X_{27} is any polar amino acid, but is preferably selected from glutamic acid, glutamine, aspartic acid, asparagine, or as discussed above cysteine. The presence of glutamic acid at position X_{27} decreases binding to IR but has less of an effect on binding to IGF-1R. X_{31} is any aromatic amino acid and X_{32} is any
- 30 small amino acid. For binding to IGF-1R, glycine or serine are preferred at position X_{31} , however, tryptophan is highly preferred for binding to IR. At

position X₃₂, glycine is preferred for both IGF-1R and IR binding. X₃₆ is an aromatic amino acid. A preferred consensus sequence for F8 is X₂₂ LC X₂₅ X₂₆ E X₂₈ X₂₉ X₃₀ WG X₃₃ X₃₄ X₃₅ X₃₆ X₃₇ X₃₈ C X₄₀ X₄₁ whereas the amino acids are defined above. A more preferred F8 sequence is

- 5 HLCVLEELFWGASLFGYCSG ("F8"). Amino acid sequences comprising the F8 sequence motif preferably bind to IR over IGF-1R. Figures 4A-4E list nonlimiting examples of Formula 4 amino acid sequences.

5. X₄₂ X₄₃ X₄₄ X₄₅ X₄₆ X₄₇ X₄₈ X₄₉ X₅₀ X₅₁ X₅₂ X₅₃ X₅₄ X₅₅ X₅₆ X₅₇ X₅₈ X₅₉ X₆₀ X₆₁ ("mini F8", Formula 5) wherein X₄₂, X₄₃, X₄₄, X₄₅, X₅₃, X₅₅, X₅₆, X₅₈, X₆₀ and X₆₁ are any amino acid. X₄₃, X₄₆, X₄₉, X₅₀ and X₅₄ are hydrophobic amino acids, however, X₄₃ and X₄₆ are preferably leucine, whereas X₅₀ is preferably phenylalanine or tyrosine but most preferably phenylalanine. X₄₇ and X₅₉ are cysteines. X₄₈ is preferably a polar amino acid, i.e. aspartic acid or glutamic acid, but most preferably glutamic acid.
- 15 Use of the small amino acid at position 54 may confer IGF-1R specificity. X₅₁, X₅₂ and X₅₇ are small amino acids, preferably glycine. A preferred consensus sequence for mini F8 is X₄₂ X₄₃ X₄₄ X₄₅ LCEX₄₉ FGGX₅₃ X₅₄ X₅₅ X₅₆ GX₅₈ CX₆₀ X₆₁. Amino acid sequences comprising the sequence of Formula 5 preferably bind to IGF-1R or IR. Nonlimiting examples of
- 20 Formula 5 amino acid sequences are described in Figure 5.

6. X₆₂ X₆₃ X₆₄ X₆₅ X₆₆ X₆₇ X₆₈ X₆₉ X₇₀ X₇₁ X₇₂ X₇₃ X₇₄ X₇₅ X₇₆ X₇₇ X₇₈ X₇₉ X₈₀ X₈₁ (Formula 6, "D8") wherein X₆₂, X₆₅, X₆₈, X₆₉, X₇₁, X₇₃, X₇₆, X₇₇, X₇₈, X₈₀ and X₈₁ may be any amino acid. X₆₆ may also be any amino acid, however, there is a strong preference for glutamic acid. Substitution of
- 25 X₆₆ with glutamine or valine may result in attenuation of binding. X₆₃, X₇₀, and X₇₄ are hydrophobic amino acids. X₆₃ is preferably leucine, isoleucine, methionine, or valine, but most preferably leucine. X₇₀ and X₇₄ are preferably valine, isoleucine, leucine, or methionine. X₇₄ is most preferably valine. X₆₄ is a polar amino acid, more preferably aspartic acid or glutamic
- 30 acid, and most preferably glutamic acid. X₆₇ and X₇₅ are aromatic amino acids. Whereas tryptophan is highly preferred at X₆₇, X₇₅ is preferably

tyrosine or tryptophan but most preferably tyrosine. X_{72} and X_{79} are cysteines which again are believed to form a loop which position amino acid may be altered by shifting the cysteines in the amino acid sequence. D8 is most useful as an amino acid sequence having a preference for binding to

- 5 IR as only a few D8 sequences capable of binding to IGF-1R over background have been detected. A preferred sequence for binding to IR is X_{62} L X_{64} X_{65} X_{66} W X_{68} X_{69} X_{70} X_{71} C X_{73} X_{74} X_{75} X_{76} X_{77} X_{78} C X_{80} X_{81} . Nonlimiting examples of Formula 6 amino acid sequences are described in Figures 6A-6E.

- 10 7. HX_{82} , X_{83} , X_{84} X_{85} X_{86} X_{87} X_{88} X_{89} X_{90} X_{91} X_{92} (Formula 7) wherein X_{82} is proline or alanine but most preferably proline; X_{83} is a small amino acid more preferably proline, serine or threonine and most preferably proline; X_{84} is selected from leucine, serine or threonine but most preferably leucine; X_{85} is a polar amino acid preferably glutamic acid, serine, lysine or
15 asparagine but more preferably serine; X_{86} may be any amino acid but is preferably a polar amino acid such as histidine, glutamic acid, aspartic acid, or glutamine; X_{87} is an aliphatic amino acid preferably leucine, methionine or isoleucine and most preferably leucine; amino acid X_{88} , X_{89} and X_{90} may be any amino acids; X_{91} is an aliphatic amino acid with a strong preference for
20 leucine as is X_{92} . Phenylalanine may also be used at position 92. A preferred consensus sequence of Formula 7 is HPPLSX₈₆LX₈₈X₈₉X₉₀LL. The Formula 7 motif binds to IR with little or no binding to IGF-1R. Nonlimiting examples of Formula 7 amino acid sequences are described in Figure 7.

- 25 8. Another sequence is X_{104} , X_{105} X_{106} X_{107} X_{108} X_{109} X_{110} X_{111} X_{112} X_{113} X_{114} . (Formula 8) which comprises eleven amino acids wherein at least one, and preferably two of the amino acids of X_{106} through X_{111} are tryptophan. In addition, it is also preferred that when two tryptophan amino acids are present in the sequence they are separated by three amino acids,
30 which are preferably, in sequential order proline, threonine and tyrosine with proline being adjacent to the tryptophan at the amino terminal end.

Accordingly, the most preferred sequence for X_{107} X_{108} X_{109} X_{110} X_{111} is WPTYW. At least one of the three amino acids on the amino terminal (X_{104} , X_{105} X_{106}) and at least one of the amino acids carboxy terminal (X_{112} X_{113} X_{114}) ends immediately flanking X_{107} - X_{111} are preferably a cysteine residue, most preferably at X_{105} and X_{113} respectively. Without being bound by theory, the cysteines are preferably spaced so as to allow for the formation of a loop structure. X_{104} and X_{114} are both small amino acids such as, for example, alanine and glycine. Most preferably, X_{104} is alanine and X_{114} is glycine. X_{105} may be any amino acid but is preferably valine. X_{112} is preferably asparagine. Thus, the most preferred sequence is ACVWPTYWNCG. The IR binding displayed amino acid sequences are described in Figure 8.

9. An amino acid sequence comprising DYKDLQSWGVRIGWLAGLCPKK (Formula 9, JBA5). The Formula 9 motif is another motif believed to form a cysteine loop which possesses agonist activity at both IR and IGF-1R. Although IR binding is not detectable by ELISA, binding of Formula 9 to IR is competed by insulin and is agonistic. See Figure 11A. Binding of Formula 9 through IGF-1R is detected by ELISA. Nonlimiting examples of Formula 9 amino acid sequences are described in Figures 9A-9C.

10. WX_{123} GYX_{124} WX_{125} X_{126} (Formula 10, Group 6 Secondary Library) wherein X_{123} is selected from proline, glycine, serine, arginine, alanine or leucine, but more preferably proline; X_{124} is any amino acid, but preferably a charged or aromatic amino acid; X_{125} is a hydrophobic amino acid preferably leucine or phenylalanine, and most preferably leucine. X_{126} is any amino acid, but preferably a small amino acid. Nonlimiting examples of Formula 10 amino acid sequences are described in Figures 10A-10B.

11. Other Motifs

Another motif for use with this invention includes WPGY. Examples of specific peptide sequences comprising this motif include KVRGFQGGTVWPGYEWLRNAKK (E8), and

KSMFVAGSDRWPGYGVADWLKK (F2).

Various amino acid sequences which bind IR and/or IGF-1R have been identified through panning of various libraries designed to identify preferred IR or IGF-1R consensus sequences which do not correspond to one of the motifs described above. Such sequences are described in Figures 10C-10I.

**B. Amino And Carboxyl Terminal Extensions
Modulate Activity of Motifs**

In addition to the motifs stated above, the invention also provides preferred sequences at the amino terminal or carboxyl terminal ends which are capable of enhancing binding of the motifs to either IR, IGF-1R, or both. In addition, the use of the extensions described below does not preclude the possible use of the motifs with other substitutions, additions or deletions which allow for binding to IR, IGF-1R or both.

1. Formula 1

Any amino acid sequence may be used for extensions of the amino terminal end of A6, although certain amino acids in amino terminal extensions may be identified which modulate activity. Preferred carboxy terminal extensions for A6 are A6 X₉₃ X₉₄ X₉₅ X₉₆ X₉₇ wherein X₉₃ may be any amino acid, but is preferably selected from the group consisting of alanine, valine, aspartic acid, glutamic acid, and arginine, and X₉₄ and X₉₇ are any amino acid; X₉₅ is preferably glutamine, glutamic acid, alanine or lysine but most preferably glutamine. The presence of glutamic acid at X₉₅ however may confer some IR selectivity. Further, the failure to obtain sequences having an asparagine or aspartic acid at position X₉₅ may indicate that these amino acids should be avoided to maintain or enhance sufficient binding to IR and IGF-1R. X₉₆ is preferably a hydrophobic or aliphatic amino acid, more preferably leucine, isoleucine, valine, or tryptophan but most preferably leucine. Hydrophobic residues, especially tryptophan at X₉₆ may be used to enhance IR selectivity.

2. Formula 2

B6 with amino terminal and carboxy terminal extensions may be represented as $X_{98} X_{99} B6 X_{100}$. X_{98} is optionally aspartic acid and X_{99} is independently an amino acid selected from the group consisting of glycine, glutamine, and proline. The presence of an aspartic acid at X_{98} and a proline at X_{99} is associated with an enhancement of binding for both IR and IGF-1R. A hydrophobic amino acid is preferred for the amino acid at X_{100} , an aliphatic amino acid is more preferred. Most preferably leucine, for IR and valine for IGF-1R. Negatively charged amino acids are preferred at both the amino and carboxy terminals of Formula 2A.

3. Formula 3

An amino terminal extension of Formula 3 defined as $X_{101} X_{102} X_{103}$ revB6 wherein X_{103} is a hydrophobic amino acid, preferably leucine, isoleucine or valine, and X_{102} and X_{101} are preferably polar amino acids, more preferably aspartic acid or glutamic acid may be useful for enhancing binding to IR and IGF-1R. No preference is apparent for the amino acids at the carboxy terminal end of Formula 3.

C. Secondary Structure

Without being bound by theory, it is believed that the B6 and reverse B6 motifs participate in alpha helix formation such that the most highly preferred residues at positions X_6, X_7, X_{10} and X_{13} (B6) and X_{14}, X_{17}, X_{20} and X_{21} (rB6) reside on the same side of a helix. See Figure 12. Because both B6 and RB6 motifs form structurally analogous motifs from their palindrome sequences, the use of D-amino acids instead of typical L-amino acids would be expected to produce amino acid sequences having similar properties to the L-amino acid sequences. D-amino acids may be advantageous, as the resultant sequences may be more resistant to enzymatic degradation than L-amino acid sequences. In addition, to maintain the appropriate orientation of highly preferred amino acid sequences on the appropriate side of the

helix, it is important to maintain the spacing of those residues along the amino acid sequence. For example, the second and third amino acids of B6 (X_7 and X_8) are oriented at opposite sides of the helix. See Figure 12.

D. IR Binding Preferences

- 5 As indicated above, the amino acid sequences containing the motifs of this invention may be constructed to have enhanced selectivity for either IR or IGF-1R by choosing appropriate amino acids at specific positions of the motifs or the regions flanking them. By providing amino acid preferences for IR or IGF-1R, this invention provides the means for constructing amino acid
- 10 sequences with minimized activity at the non-cognate receptor. For example, the amino acid sequences disclosed herein with high affinity and activity for IR and low affinity and activity for IGF-1R are desirable as IR agonist as their propensity to promote undesirable cell proliferation, an activity of IGF-1 agonists, is reduced. Ratios of IR binding affinity to IGF-1R
- 15 binding affinity for specific sequences are provided in Figures 1A-10I. As an insulin therapeutic, the IR/IGF-1R binding affinity ratio is preferably greater than 100. Conversely, for use as an IGF-1R therapeutic, the IR/IGF-1R ratio should be less than 0.01. Examples of peptides that selectively bind to IGF-1R are shown below.

20

IGF-1R-SELECTIVE SEQUENCES

MOTIF 1 (A6-like):

Clone	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGF-1R	IGF-1R/IR	IR/IGF-1R
A6L-0-E6-IR	YRGMLVLGRSSDGAGKVAEPARIQQTFAVNFYDNFV	31.0	31.0	17.0	0.1
H2CA-4-G9-IGFR	GIISQCPSEFYDNFAGQVSDPWCV	8.6	9.5	16.0	0.1
H2CA-4-H6-IGFR	VGRASGFPENFYDNFGRQLSLQSGEQ	4.9	10.5	14.6	0.1
A6L-0-E4-IR	YRGMLVLGRISDGAG#VASEPPARIGRKYFVNFYDNFV	26.0	16.0	13.0	0.1
A6L-0-E4-IR	YRGMLVLGRISDGAGKAAERPARIGQKVSANFYDNFV	27.0	26.0	13.0	0.1
A6L-0-H3-IR	VGQGGDENFYDNFIRQVSGRLGVQ	5.5	9.7	12.3	0.1
H2CA-4-F5-IGFR	SACQFDCHEFYDNFARQVSGGAAYG	5.6	9.2	9.4	0.1
H2CA-4-H8-IGFR	SAQQLFFQESFYDNFIRQVAESSQPN	3.5	6.8	6.7	0.1
H2CA-4-F11-IGFR	AVRATRDEAFYDNFVRQISDGQGNK	3.9	7.3	6.4	0.2
H2CA-4-F6-IGFR	VNOSGSIHENFYDNFVRQVSRQGRV	4.9	5.7	5.9	0.2
H2CA-4-F10-IGFR	APDSPQIEIFYDNFVRQVSRMPGGG	7.7	3.8	5.1	0.2
H2CA-1-A3-IGFR	SSCDGAGHESFYDNFVRQVSGCRSV	15.1	5.6	1.2	4.8
H2CA-3-C8-IGFR	RAGSSDPHEDFYDNFVRQVSLKGGK	9.3	7.0	4.2	0.2
H2CA-2-B9-IGFR	QAVQGFHEBFYDNFVRQVSTGVGGG	3.9	4.1	4.2	0.2
H2CA-4-H4-IGFR	GFRGNFYENFQAQVOT	37.8	33.9	4.1	0.2
E4Da-4-H2-IR	SSI GGGFHENFYDNFISQSLQSPPLK	1.5	3.2	4.1	0.2
H2CA-4-F7-IGFR	QSPVSSSHEDFYDNFIRQVAQSGAHQ	8.3	9.0	4.0	0.3
H2CA-3-D6-IGFR	NYRQVFNNGFYDNFDRQVFSLVTPG	10.9	7.2	4.0	0.3
H2CA-3-D8-IGFR	TLDGGSFEEQFYDNFVRQLSYRTNPD	10.8	9.5	3.9	0.3
H2CA-4-G11-IGFR	FYVQWGHENFYDNFDRQVSGGGAG	5.8	3.5	3.8	0.3
H2CA-4-F1-IGFR	LRRQAPVEHENFYDNFVRQVSGDRVGG	13.3	3.0	3.7	0.3
H2CA-3-D7-IGFR	RCGRELXHSIFYDNFDRQVAGRTCPG	8.0	2.2	3.7	0.3
H2CA-1-A7-IGFR	CCLLCRFQNFYDNFVCCQGISLRLPL	3.5	4.1	3.6	0.3
H2CA-2-B4-IGFR	PPLASDLVDQFYGNFVQVQVSPPPGGG	7.7	3.8	3.6	0.3
H2CA-2-B3-IGFR					

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Clone	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGF-1R	IGF-1R/IR	IR/IGF-1R
H2CA-2-B2-IGFR	GAPVDQLHEDFYDWFVRQVSQAATG	4.1	3.4	3.5	0.3
E4Dα-2-D11-IR	GFREGSFYDWFQAQVT	40.2	11.1	3.4	0.3
20E2Bβ-4-G6-IR	SQAGSAFYAWFDQVLRIVHSA	22.4	6.2	3.3	0.3
H2CA-4-H9-IGFR	RGAVAGFHDQFYDWFDRQVSRVHKFG	8.7	5.6	3.0	0.3
H2CA-2-B11-IGFR	AICDAGFHEHFYDWFALQVSDCGRQS	11.9	4.6	3.0	0.3
H2CA-3-E8-IGFR	LGYOEPFQNFYDWFVRQVSGAENAG	13.2	6.3	2.9	0.3
A6S-2-D11-IR	EAASLGSQDRNFYDWFVRQV	48.4	37.4	2.8	0.4
A6S-3-E2-IR	VERSASSQDNFYDWFVQVIR	37.8	30.6	2.6	0.4
H2CA-3-E11-IGFR	TSEVQRSSQDNFYDWFVAOVA	33.1	24.7	2.5	0.4
H2CA-3-C11-IGFR	HLADAGFHEKFYDWFERQISSRCNDIC	4.7	2.2	2.2	0.5
	FRTLAAQHDSFYDWFDRQVSGAAGER	9.3	3.3	2.1	0.5
A6-PD1-IGFR	SFHEDFYDWFDRQVSGSLKK				
H2C-PD1-IGFR (RP9)	GSILDESFYDWFERQLGKK				

MOTIF 2 (B6-like):

Clone	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGF-1R	IGF-1R/IR	IR/IGF-1R
20C-3-G3-IGFR	TFYSCIASLLTGTQPNRGPWRCR	33.1	32.3	1.2	27.0
20C-4-C7-IGFR	FFYDCLAAQLQGVARYHDLCAVEIT	35.3	28.0	1.3	21.8
B6H α -1-B5-IR	CTTEMVMDARDPFFVHKLSELVTGG	41.5	20.5	1.0	20.5
R20B-4-A6-IR	RGSDAFYSGLWALIGLSDG	9.3	25.9	1.5	17.3
20E2B-1-A6-IGFR	GVRAMSFYDALVSVLIGLPGSG	18.6	18.1	1.1	16.8
R20 α -4-20A12-IR	RLFYCGIQALGANLIGYSGCV	48.6	39.9	2.4	16.6
20E2B β -4-G7-IR	LQPCSGFYECTERLIGVKLSG	19.9	25.2	1.6	15.8
NNRPY-4-B11-IR	LKDGFDYFWQRLHLS	4.1	18.7	1.2	15.5
20E2B-3-C6-IGFR	VEGRGLFYDLLRLQLARRQNG	17.9	16.8	1.1	14.8
B6H α -1-A2-IR	RGCNDDGGKGGDDPFFVHKLSELICGG	22.3	14.6	1.0	14.6
20E2A-4-F11-IGFR	QGGASFYDAIDRLRMRIIG	21.3	18.8	1.3	14.6
B6H α -3-E9-IR	RCEEKQAEVGPSPDPFFVHKLSELGCR	44.6	24.2	1.7	14.2
20C-3-F6-IGFR	DRDFCFYERLTALVGGQVDGWNPC	33.5	26.1	1.9	14.1
20E2B-4-H3-IGFR	KLHNLMFYIGLRLVWAGL	11.2	14.8	1.1	13.9
20E2B-3-C2-IGFR	GNGDGMFYQLLSLVGRDMHV	13.1	8.9	0.6	13.8
20C-3-A1-IGFR	SSYCGDGFYLMFLSLGVASQELLEC	26.5	20.8	1.5	13.7
20E2B-3-E3-IGFR	PDLHKGFYAQLAQLIRGQLLS	22.4	16.3	1.3	13.1
R20 α -3-20E2-IR	FYDAIDQLVRSARAGGTDR	46.3	39.9	3.1	12.9
20E2B-4-H12-IGFR	YSCGDGFYLLSLLLGQFRC	6.5	9.7	0.8	12.8
B6H α -3-F11-IR	RGMKREVLVGGSTDPFFVHKLSELQGS	49.5	18.7	1.6	11.7
20E2B-3-D2-IGFR	IQQELTFYDLLHRLVRSELGS	20.7	12.4	1.1	11.7
20E2B-3-D8-IGFR	GGTEVDFFYRALERLVRQGL	20.4	17.7	1.6	11.3
20E2B-3-E8-IGFR	LRIANLIFYORLWDIAFGGG	15.7	16.7	1.5	11.1
B6H α -2-C4-IR	RCGRW*AEWGAGDDPFFVHKLSELVCG	20.7	9.9	0.9	11.0
R20 α -4-20C11-IR	DRAFTNGRLDVLGVAVGAWD	43.7	30.8	3.0	10.3
20E2B-4-F8-IGFR	PVGQGFYEGLSRLVIRGGW	12.3	7.3	0.8	9.7

Clone	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGF-1R	IGF-1R/IR	IR/IGF-1R
B6H α -3-E8-IR	RGKTAIVIVGRPADPFYHKLSELLQGG	47.6	5.3	1.1	4.8
B6H α -3-F10-IR	GCVEWQKHGASDPFYHKLSELLQGG	47.2	8.8	1.9	4.6
B6H α -2-D6-IR	GRTMAVMAAGPDDPFYHKLSELLQGG	33.5	4.4	1.0	4.4
B6H α -3-E7-IR	GCAVVEAERSGDPFYHKLSELLQGG	47.0	5.6	1.3	4.3
B6H α -2-D1-IR	GCEVIEEGSDAPFYHKLSELLQGG	11.7	5.4	1.3	4.2
20E2A-3-D10-IGFR	MMVVDGFYDALHQLVVAQSLG	20.6	6.9	1.8	3.9
20E2A-3-A12-IGFR	LSVALSFYDALQLVAGEGRW	16.1	4.3	1.1	3.9
B6H α -4-G8-IR	GGTKAVAKVTRDDPFYHKLSELLQGS	32.3	6.1	1.7	3.6
B6L-4-D7-IR	AETSVQVGIQLQSVWPGHEWNTVDPFYHKLSELLRSGA14.3	4.8	1.4	0.3	0.3
B6H α -1-A3-IR	SRAKVEAMPDSDGDPFYHKLSELLASG	37.4	2.6	0.8	3.3
B6H α -3-F7-IR	SRVAATKEKRPDDPFYHKLSELLQGS	41.5	3.1	1.0	3.1
B6H α -2-D8-IR	SSETAKWVTGTRDDPFYHKLSELLQGS	19.3	3.0	1.0	3.0
B6H α -1-B3-IR	GCITAENGAGDPPFYHKLSELLQGS	33.1	3.2	1.1	0.3
B6H α -3-E5-IR	RCGDEEGWQENRRDDPFYHKLSELLFGG	28.8	2.9	1.0	2.9
20E2A-4-G11-IGFR	MNVFVSFYDALQLVQCRIG	20.7	3.3	1.3	2.6
20E2B β -3-C7-IR	QSGSGDFYDWLSRLIRNGDG	1.5	3.1	1.5	2.0
B6H α -3-E6-IR	CGAKWITGPNDDPFYHKLSELLQRG	18.2	2.3	1.2	1.9
20E2A-3-A3-IGFR	GHYFGSFYDALQLVAGMLPG	5.2	3.0	1.5	0.5
B6L-4-A7-IR	AGTAPQVQ*NRKLWSVWPGHEWNTVDPFYHKLSELLRESG11.6	3.4	3.4	1.9	1.8
B6H α -3-F1-IR	CSMAVAEAGDDDDPFYHKLSELLQGS	22.5	2.4	1.3	1.8
B6L-3-G6-IR	VDTPAQVGNRLVSGPGEHWTDDPFYH*LSPELLRESGA7.6	2.5	2.5	1.8	1.4
B6L-3-G5-IR	AETSAQVGNQRLWSVWPGDHWSTLDDPFYHKLSELLRESG11.5	2.0	2.0	1.4	0.7
20E2A-3-A4-IGFR	AGSVTSFYDAMEQLVATGTS	16.8	2.5	1.8	1.4

B6-PD1-IGFR TDDGFYDALEQLVQSGKK
20E2-PD1-IGFR (RP10) GSFYEAQLRVGGEQGKK

Besides relative binding at IR or IGF-1R, relative efficacy at the cognate receptor is another important consideration for choosing a potential therapeutic. Thus, a sequence which is efficacious at IR but has little or no significant activity at IGF-1R may also be considered as an important IR therapeutic, irrespective of the relative binding affinities at IR and IGF-1R.

A6 selectivity for IR may be enhanced by including glutamic acid in a carboxyl terminal extension at position X₉₅. IR selectivity of the B6 motif may be enhanced by having a tryptophan or phenylalanine at X₁₁. Tryptophan at X₁₃ also favors selectivity of IR. A tryptophan amino acid at X₁₃ rather than leucine at that position also may be used to enhance selectivity for IR. In the reverse B6 motif, a large amino acid at X₁₅ favors IR selectivity. Conversely, small amino acids may confer specificity for IGF-1R. In the F8 motif, an L in position X₂₃ is essentially required for IR binding. In addition, tryptophan at X₃₁ is also highly preferred. At X₃₂, glycine is preferred for IR selectivity.

E. Multiple Binding Sites On IR And IGF-1R

The competition data disclosed herein reveals that at least two separate binding sites are present on IR and IGF-1R which recognize the different sequence motifs provided by this invention.

As shown in Figure 13, competition data (See Example 15) indicates that peptides comprising the A6, B6, revB6, and F2 motifs compete for binding to the same site on IR (Site 1) whereas the F8 and D8 motifs compete for a second site (Site 2). Similarly, the decrease of dissociation of B6 motif peptide (20E2) from IGF-1R by a D8 ligand indicates multiple interacting binding sites.

The identification of peptides which bind to separate binding sites on IR and IGF-1R provides for various schemes of binding to IR or IGF-1R to increase or decrease its activity. Examples of such schemes for IR are illustrated in Figure 15.

The table below shows sequences based on their groups, which bind to Site 1 or Site 2.

REPRESENTATIVE SITE 1 PEPTIDES

A6-like (FYxWF):

	Clone	Sequence
5	G3	KRGGGTFYEFWFESALRKHGAGKK
	H2	VTFTSAVFHENFYDWFVRQVSKK
	H2C	FHFNFYDWFVRQVSKK
	A6S-IR3-E12	GRVDWLQRNANFYDWFVAELG
	A6S-IR4-G1	NGVERAGTGDNFYDWFVAQLH
10	H2CB-R3-B12	QSDSGTVHDFRYGNFDRDTWAS
15	20E2A-R3-B11	GRFYGNFQDAIDQLMPWGFPD
	rB6-F6	RYGRWGLAQQFYDWFDR
	E4Dα-1-B8-IR	GFREGQRWYNFVAQVT
	H2CA-4-F11-IR	TYKARFLHENFYDWFNRQVSQYFGRV
	H2CB-R3-D2	WTDVDGFHSGFYRWFPQNOWER
20	H2CB-R3-D12	VASGHVHLHGQFYRWFPDQFAL
	H2CB-R4-H5	QARVGNVHQGFYWFREVMQG
	H2C-B-E8*	TGHRLLGLDEQFYWWFRDALS
	H2CB-3-B6-IR	VGDPCVSHDCFYGNFLRESMQ
	A6S-IR2-C1	RMFYSTGAPQNFYDWFVQEW

B6-like (FYxxLxxL):

	Clone	Sequence
25	20C11	KDRAFYNGLRDLVGAVYGAWDKK
	20E2	DYKDFYDAIDQLVRGSARAGGTRDKK
	B62-R3-C7	EHWNTVDPFYFTLFEWLRESG
	B62-R3-C10	EHWNTVDPFYQYFSELLRESG
30	20E2B-3-B3-IR	AGVNAGFYRYFSTLLDWDQDQ
	20E2-B-E3*	IQGWEFPYGNWFDVVAQMFEE
	20E2A-R4-F9	PPWGARFYDAIEQLVFDNLCC
	RFNN-4-G6-HOLO*	RWPNFYGYFESLLTHFS
35	RFNN-4-F3-HOLO*	HYNFYGYFQVLLAETW
	20E2A-R4-E2	IGRVRSFYDAIDKLQSDWER
	RFNN-2-C1-IR*	EGWDFYSYFSGLLASVT
	20E2B-4-F12-IR	SVKEVQFYRYFYDQLQSEESG
	20E2-B-E12	GNSGGSFYRYFQLLDSDGMS
	20E2A-R3-B6	RDAGSSFYDAIDQLVCLTYFC

Reverse B6-like (LxxLxxYF):

Clone	Sequence
rB6-A12	LDALDRLMRYFEERPSSL
rB6-F9	PLAELWAYFEHSEQGRSSAH
rB6-4-E7-IR	LDPLDALLQYFWSVPGH
rB6-4-F9-IR	RGRGLGSLSTQFYWNFAE
rB6-B6	ADELEWLLDYFMHQPRP
rB6-4-F12-IR	DGVLEELFSYFSATVGP

Group 6 (WPxYxWL):

Clone	Sequence
R20β-4-A4-IR	WPGYLFEEALQDWRGSTED

Peptides by design**:

Clone	Sequence
H2C-PD1-IR~	AAVHEQFYDWFADQYKK
A6S-PD1-IR~	QAPSNFYDWFVREWDKK
20E2-PD1-IR~	QSFYDYIEELLGGGEKK
60 B6C-PD1-IR~	DPFYQGLWEWLRESGKK

REPRESENTATIVE SITE 2 PEPTIDES (C-C LOOPS)

F8-derived (Long C-C loop):

	Clone	Sequence
5	F8	HLCVLEELFWGASLFGYCSG
	F8-C12	FQSLLEELVWGAPLFRYGTG
	F8-Des2	PLCVLEELFWGASLFGYCSG
10	F8-F12	PLCVLEELFWGASLFGQCSCG
	F8-B9	HLCVLEELFWGASLFGQCSCG
	F8-B12	DLRVLCLELFGGAYVLGYCSE
15	NNKH-2B3	HRSVLKQLSWGASLFGQWAG
	NNKH-2F9-	HLSVGEELSWVALLGQWAR
	NNKH-4H4-	APVSTELRWGALLFGQWAG

D8-derived (Small C-C loop):

	Clone	Sequence
20	D8	KNLDQEWAWVQCEVYGRGCPSSK
	D8-G1	QLEEWAGVQCEVYGRGCPSS
	D8-B5-	ALBEWAWVQVRSIRSGPL
	D8-A7	SLDQEWAWVQCEVYGRGCLS
25	D8-F1-	WLEHEWAQIQCELYGRGCTY

Midi C-C loop:

	Clone	Sequence
30	D8-F10	GLEQGCPRVGLGVQCRGCPSS
	F8-B12~	DLRVLCLELFGGAYVLGYCSE
	F8-A9	PLWGLCELFGGASLFGYCSC

**Based on analysis of entire panning data, amino acid preferences at each position were calculated to define these "idealized" peptides.

35 * Peptides synthesized and currently being purified

~ Peptides planned

F. Multivalent Ligands

This invention provides ligands which preferentially bind different
 40 sites on IR and IGF-1R. The amino acid motifs which bind IR at one site
 (Site 1, Figure 13) are A6, B6, revB6, and F2. A second in site (Site 2,
 Figure 13) binds F8 and D8. Accordingly, multimeric ligands may be
 prepared according to the invention by covalently linking amino acid
 sequences. Depending on the purpose intended for the multivalent ligand,
 45 amino acid sequences which bind the same or different sites may be
 combined to form a single molecule. Where the multivalent ligand is
 constructed to bind to the same corresponding site on different receptors, or

different subunits of a receptor, the amino acid sequences of the ligand for binding to the receptors may be the same or different, provided that if different amino acid sequences are used, they both bind to the same site.

- 5 Multivalent ligands may be prepared by either expressing amino acid sequences which bind to the individual sites separately and then covalently linking them together, or by expressing the multivalent ligand as a single amino acid sequence which comprises within it the combination of specific amino acid sequences for binding.

- 10 Various combinations of amino acid sequences may be combined to produce multivalent ligands having specific desirable properties. Thus, agonists may be combined with agonists, antagonists combined with antagonists, and agonists combined with antagonists. Combining amino acid sequences which bind to the same site to form a multivalent ligand may be useful to produce molecules which are capable of cross-linking together
- 15 multiple receptor units. Multivalent ligands may also be constructed to combine amino acid sequences which bind to different sites (Figure 15).

- In view of the discovery disclosed herein of monomers having agonist properties at IR or IGF-1R, preparation of multivalent ligands may be useful to prepare ligands having more desirable pharmacokinetic properties due to the presence of multiple bind sites on a single molecule. In addition,
- 20 combining amino acid sequences which bind to different sites with different affinities provides a means for modulating the overall potency and affinity of the ligand for IR or IGF-1R.

1. Construction of Hybrids

- 25 In one embodiment, hybrids of at least two peptides may be produced as recombinant fusion polypeptides which are expressed in any suitable expression system. The polypeptides may bind the receptor as either fusion constructs containing amino acid sequences besides the ligand binding sequences or as cleaved proteins from which signal sequences or other
- 30 sequences unrelated to ligand binding are removed. Sequences for facilitating purification of the fusion protein may also be expressed as part of

the construct. Such sequences optionally may be subsequently removed to produce the mature binding ligand. Recombinant expression also provides means for producing large quantities of ligand. In addition, recombinant expression may be used to express different combinations of amino acid sequences and to vary the orientation of their combination, i.e., amino to carboxyl terminal orientation.

Whether produced by recombinant gene expression or by conventional chemical linkage technology, the various amino acid sequences may be coupled through linkers of various lengths. Where linked sequences are expressed recombinantly, and based on an average amino acid length of about 4 angstroms, the linkers for connecting the two amino acid sequences would typically range from about 3 to about 12 amino acids corresponding to from about 12 to about 48 Å. Accordingly, the preferred distance between binding sequences is from about 2 to about 50 Å. More preferred is 4 to about 40. The degree of flexibility of the linker between the amino acid sequences may be modulated by the choice of amino acids used to construct the linker. The combination of glycine and serine is useful for producing a flexible, relatively unrestrictive linker. A more rigid linker may be constructed by using amino acids with more complex side chains within the linkage sequence.

In a preferred embodiment shown below (Figure 16)

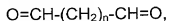
MBP-FLAG-PEPTIDE-(G,S)_n-PEPTIDE-E-TAG

a fusion construct producing a dipeptide comprises a maltose binding protein amino acid sequence (MBP) or similar sequence useful for enabling the affinity chromatography purification of the expressed peptide sequences. This purification facilitating sequence may then be attached to a flag sequence to provide a cleavage site to remove the initial sequence. The peptide dimer then follows which includes the intervening linker and a tag sequence may be included at the carboxyl terminal portion to facilitate identification/purification of the expression of peptide. In the representative

construct illustrated above, G and S are glycine and serine residues, which make up the linker sequence.

- In addition to producing the dimer peptides by recombinant protein expression, dimers may also be produced by peptide synthesis whereby a synthetic technique such as Merrifield synthesis (Merrifield, 1997), may be used to construct the entire peptide.

- Other methods of constructing dimers include introducing a linker molecule which activates the terminal end of a peptide so that it can covalently bind to a second peptide. Examples of such linkers include diamino propionic acid activated with an oxyamino function. A preferred linker is a dialdehyde having the formula



- Wherein n is 2 to 6, but is preferably 6 to produce a linker of about 25 to 30 angstroms in length. Linkers may be used to link dimers either to the carboxyl terminal or the amino terminal.

2. Characterization Of Specific Dimers

- Specific dimers which bind with high affinity to Site 1, Site 2, or both Site 1 and Site 2 of the insulin receptor are shown in Table 1. Although agonist activity has been observed for the Site 1-Site 1 dimers, the Site 1-Site 2 dimers may also possess desirable properties.

TABLE 1

Fusion	Seq.	Action	Site	Fusion Concentration	MW (kDa)	K _d (HIR)
426	D8	N	2	0.76	52.2	1.4 x 10 ⁻⁶
429	D8-6aa-D8	N-N	2-2	3.2	55.3	1.3 x 10 ⁻⁶
430	H2C-6aa-RB6	A-	1-1	0.17	54.5	2.1 x 10 ⁻⁶
431	H2C-6aa-F8	A-N	1-2	3.3	54.8	4.7 x 10 ⁻⁸
432	H2C-12aa-F8	A-N	1-2	2.9	55.5	3.5 x 10 ⁻⁸
433	H2C-9aa-F8	A-N	1-2	2.8	55.2	2.1 x 10 ⁻⁸
434	G3-12aa-G3	N-N	1-1	0.01	56	3.2 x 10 ⁻⁶
436	H2C-9aa-H2C	A	1-1	1.1	54.2	4.1 x 10 ⁻⁷
437	H2C	N-N	1	0.3	51.5	8.3 x 10 ⁻⁶
427	G3-6aa-G3	N-N	1-1	0.02	55.3	3.3 x 10 ⁻⁶
435	H2C-3-H2C-3-H2C	A-A-A	1-1-1	2.1	55.5	2.0 x 10 ⁻⁶
439	H2C-6aa-H2C	A-A	1-1	1.4	53.9	5.5 x 10 ⁻⁷
449	H2C-12aa-H2C		1-1	1.5	51.8	6.2 x 10 ⁻⁷
452	G3		1	0.15	48.8	7.8 x 10 ⁻⁷
463	H2C-3aa-H2C	A-A	1-1	1.8	50.1	9.6 x 10 ⁻⁷
464	LF-H2C		1	0.045	48.4	3.9 x 10 ⁻⁸
446	LF-F8		2	1.9	49.1	7.7 x 10 ⁻⁷
459	SF-RB6			0.069	48.1	7.7 x 10 ⁻⁸
MBP*	lacZ			5.1	50	> 1 x 10 ⁻⁵

*MBP (negative control for the fusions) is fused to a small fragment of beta-galactosidase (lacZ).

N = Antagonist

A = Agonist

LF = Long FLAG epitope (DYKDDDDK)

SF = Short FLAG epitope (DYKD)

Additional binding data for the fusion peptides are shown below:

Fusion	Highest conc. tested (μM)	K _d (HIR) μM
431-	0.2	0.033
431+	0.2	0.0074
432-	0.2	0.02
432+	0.2	0.0038
433-	0.2	0.03
433+	0.2	0.004

The concentrations of these fusions vary depending on the expression quality.

- 5 There are 2 sets of each fusion: uncleaved (-) and cleaved with factor Xa (+). The fusion proteins are in Tris buffer (20 mM Tris, 200 mM NaCl, 1 mM EDTA, 50 mM maltose, pH 7.5) and the cleaved fusions (+) are in the same Tris buffer (500 μl) + 12 μg Factor Xa. (Source of Factor Xa: New England Biolabs).

Other combinations of peptides are within the scope of this invention and may be determined as demonstrated in the examples described herein.

Regarding preparation of a Site 1 agonist comprising two D117 (H2C) peptides, a linker of only 3 amino acids (12 Å) provided a ligand of greater
5 affinity for Site 1 of IR than a corresponding ligand prepared with a 9 amino acid (36 Å) linking region. Figure 17.

Notably, several fusion peptides show IR agonist activity as determined by an IR autophosphorylation assay (see Example 20). Figure
74. In particular, fusion peptides 439, 436, 449, and 463 show significant IR
10 agonist activity (Figure 74).

G. Peptide Synthetic Techniques

Many conventional techniques in molecular biology, protein biochemistry, and immunology may be used to produce the amino acid sequences for use with this invention.

15 1. Recombinant Synthesis

To obtain recombinant peptides, the corresponding DNA sequences may be cloned into any suitable vectors for expression in intact host cells or in cell-free translation systems by methods well known in the art (see
Sambrook *et al.*, 1989). The particular choice of the vector, host, or
20 translation system is not critical to the practice of the invention.

Cloning vectors for the expression of recombinant peptides include, but are not limited to, pUC, pBluescript (Stratagene, La Jolla, CA), pET (Novagen, Inc., Madison, WI), pMAL (New England Biolabs, Beverly, MA), or pREP (Invitrogen Corp., San Diego, CA) vectors. Vectors can contain
25 one or more replication and inheritance systems for cloning or expression, one or more markers for selection in the host (e.g. antibiotic resistance), and one or more expression cassettes. The inserted coding sequences can be synthesized by standard methods, isolated from natural sources, or prepared as hybrids, etc. Ligation of the coding sequences to transcriptional
30 regulatory elements and/or to other amino acid coding sequences can be

carried out using established methods. DNA sequences can be optimized, if desired, for more efficient expression in a given host organism. For example, codons can be altered to conform to the preferred codon usage in a given host cell or cell-free translation system using techniques routinely practiced in the art.

Suitable cell-free systems for expressing recombinant peptides include, for example, rabbit reticulocyte lysate, wheat germ extract, canine pancreatic microsomal membranes, *Escherichia coli* (*E. coli*) S30 extract, and coupled transcription/translation systems (Promega Corp., Madison, WI). Such systems allow expression of recombinant polypeptides upon the addition of cloning vectors, DNA fragments, or RNA sequences containing coding regions and appropriate promoter elements.

Host cells for cloning vectors include bacterial, archeobacterial, fungal, plant, insect and animal cells, especially mammalian cells. Of particular interest are *E. coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Neurospora crassa*, SF9, C129, 293, NIH 3T3, CHO, COS, and HeLa cells. These cells can be transformed, transfected, or transduced, as appropriate, by any suitable method including electroporation, CaCl_2 , LiCl , LiAc/PEG -, spheroplasting-, Ca-Phosphate , DEAE-dextran, liposome-mediated DNA uptake, injection, microinjection, microprojectile bombardment, or other established methods.

For some purposes, it may be preferable to produce peptides in a recombinant system in which they carry additional sequence tags to facilitate purification. Non-limiting examples of tags include c-myc, haemagglutinin (HA), polyhistidine (6X-HIS), GLU-GLU, and DYKDDDDK (FLAG®) epitope tags. Epitope tags can be added to peptides by a number of established methods. DNA sequences of epitope tags can be inserted into peptide coding sequences as oligonucleotides or through primers used in PCR amplification. As an alternative, peptide coding sequences can be cloned into specific vectors that create fusions with epitope tags; for

example, pRSET vectors (Invitrogen Corp., San Diego, CA). The expressed, tagged peptides can then be purified from a crude lysate of the cell-free translation system or host cell by chromatography on an appropriate solid-phase matrix.

- 5 Methods for directly purifying peptides from natural sources such as cellular or extracellular lysates are well known in the art (see Harris and Angal, 1989). Such methods include, without limitation, sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE), preparative disc-gel electrophoresis, isoelectric focusing, high-performance liquid
- 10 chromatography (HPLC), reversed-phase HPLC, gel filtration, ion exchange and partition chromatography, countercurrent distribution, and combinations thereof. Naturally occurring peptides can be purified from many possible sources, for example, plasma, body tissues, or body fluid lysates derived from human or animal, including mammalian, bird, fish, and insect sources.
- 15 Antibody-based methods may also be used to purify naturally occurring or recombinantly produced peptides. Antibodies that recognize these peptides or fragments derived therefrom can be produced and isolated. The peptide can then be purified from a crude lysate by chromatography on an antibody-conjugated solid-phase matrix (see Harlow
- 20 and Lane, 1998).

2. Chemical Synthesis Of Peptides

- Alternately, peptides may be chemically synthesized by commercially available automated procedures, including, without limitation, exclusive solid phase synthesis, partial solid phase methods, fragment condensation or
- 25 classical solution synthesis. The polypeptides are preferably prepared by solid-phase peptide synthesis; for example, as described by Merrifield (1965; 1997). In addition, recombinant and synthetic methods of polypeptide production can be combined to produce semi-synthetic polypeptides.

H. Screening Assays

In another embodiment of this invention, screening assays to identify pharmacologically active ligands at IR and/or IGF-1R are provided. The screening assays provided in accordance with this invention are based on those disclosed in International application WO 96/04557 which is incorporated herein in its entirety. Briefly, WO 96/04557 discloses the use of reporter peptides which bind to active sites on targets and possess agonist or antagonist activity at the target. These reporters are identified from recombinant libraries and are either peptides with random amino acid sequences or variable antibody regions with at least one CDR region which has been randomized (rVab). The reporter peptides may be expressed in cell recombinant expression systems, such as for example in *E. coli*, or by phage display. See WO 96/04557 and Kay *et al.* (1996), both of which are incorporated herein by reference. The reporters identified from the libraries may then be used in accordance with this invention either as therapeutics themselves, or in competition binding assays to screen for other molecules, preferably small, active molecules, which possess similar properties to the reporters and may be developed as drug candidates to provide agonist or antagonist activity. Preferably, these small organic molecules are orally active.

The basic format of an *in vitro* competitive receptor binding assay as the basis of a heterogeneous screen for small organic molecular replacements for insulin may be as follows: occupation of the active site of IR is quantified by time-resolved fluorometric detection (TRFD) with streptavidin-labeled europium (saEu) complexed to biotinylated peptides (bP). In this assay, saEu forms a ternary complex with bP and IR (i.e., IR:bP:saEu complex). The TRFD assay format is well established, sensitive, and quantitative (Tompkins *et al.*, 1993). The assay can use a single-chain antibody or a biotinylated peptide. Furthermore, both assay formats faithfully report the competition of the biotinylated ligands binding to the active site of IR by insulin.

5 In these assays, soluble IR is coated on the surface of microtiter wells, blocked by a solution of 0.5% BSA and 2% non-fat milk in PBS, and then incubated with biotinylated peptide or rVab. Unbound bP is then washed away and saEu is added to complex with receptor-bound bP. Upon addition of the acidic enhancement solution, the bound europium is released as free Eu^{3+} which rapidly forms a highly fluorescent and stable complex with components of the enhancement solution. The IR:bP bound saEu is then converted into its highly fluorescent state and detected by a detector such as Wallac Victor II (EG&G Wallac, Inc.)

10 The designing of mimetics to a known pharmaceutically active compound is a known approach to the development of pharmaceuticals based on a "lead" compound. This might be desirable where the active compound is difficult or expensive to synthesize or where it is unsuitable for a particular method of administration, e.g. peptides are generally unsuitable
15 active agents for oral compositions as they tend to be quickly degraded by proteases in the alimentary canal. Mimetic design, synthesis and testing is generally used to avoid randomly screening large number of molecules for a target property.

20 There are several steps commonly taken in the design of a mimetic from a compound having a given target property. First, the particular parts of the compound that are critical and/or important in determining the target property are determined. In the case of a peptide, this can be done by systematically varying the amino acid residues in the peptide (e.g. by substituting each residue in turn). These parts or residues constituting the
25 active region of the compound are known as its "pharmacophore".

Once the pharmacophore has been found, its structure is modeled according to its physical properties (e.g. stereochemistry, bonding, size and/or charge), using data from a range of sources (e.g. spectroscopic techniques, X-ray diffraction data and NMR). Computational analysis,
30 similarity mapping (which models the charge and/or volume of a

pharmacophore, rather than the bonding between atoms), and other techniques can be used in this modeling process.

In a variant of this approach, the three dimensional structure of the ligand and its binding partner are modeled. This can be especially useful where the ligand and/or binding partner change conformation on binding, allowing the model to take account of this in the design of the mimetic.

A template molecule is then selected onto which chemical groups which mimic the pharmacophore can be grafted. The template molecule and the chemical groups grafted on to it can conveniently be selected so that the mimetic is easy to synthesize, is likely to be pharmacologically acceptable, does not degrade *in vivo*, and retains the biological activity of the lead compound. The mimetics found are then screened to ascertain the extent they exhibit the target property, or to what extent they inhibit it. Further optimization or modification can then be carried out to arrive at one or more final mimetics for *in vivo* or clinical testing.

This invention provides specific IR and IGF-1R amino acid sequences which function as either agonists or antagonists at IR and/or IGF-1R. Examples of phage display libraries suitable for use in this invention include one such library containing randomized 40 amino acid peptides (RAPIDLIB™, Figure 16), another library containing rVab derived from human genomic antibody DNA (GRABLIB™, Figure 30). Details of the construction and analyses of these libraries, as well as the basic procedures for biopanning and selection of binders, have been described elsewhere (WO 96/04557; Mandecki *et al.*, 1997; Ravera *et al.*, 1998; Scott and Smith, 1990); Grihalde *et al.*, 1995; Chen *et al.*, 1996; Kay *et al.*, 1993, Carcamo *et al.*, 1998, all of which are incorporated herein by reference). Another phage display library suitable for use with this invention is available commercially from New England Biolabs (Ph.D. C7C Disulfide Constrained Peptide Library). Additional sequences may be obtained in accordance with the procedures described herein.

plasmid) to a protein ligand via polylysine. The appropriate or suitable ligands are selected on the basis of the presence of the corresponding ligand receptors on the cell surface of the target cell or tissue type. These ligand-DNA conjugates can be injected directly into the blood, if desired, and are directed to the target tissue where receptor binding and DNA-protein complex internalization occur. Co-infection with adenovirus to disrupt endosome function can be used to overcome the problem of intracellular destruction of DNA.

An approach that combines biological and physical gene transfer methods utilizes plasmid DNA of any size combined with a polylysine-conjugated antibody specifically reactive with the adenovirus hexon protein. The resulting complex is bound to an adenovirus vector. The trimolecular complex is then used to infect cells. The adenovirus vector allows efficient binding to the cell, internalization, and degradation of the endosome before the coupled DNA can be damaged.

Many types of cells and cell lines (e.g. primary cell lines or established cell lines) and tissues are capable of being stably transfected by or receiving the constructs of the invention. Examples of cells that may be used include, but are not limited to, stem cells, B lymphocytes, T lymphocytes, macrophages, other white blood lymphocytes (e.g. myelocytes, macrophages, monocytes), immune system cells of different developmental stages, erythroid lineage cells, pancreatic cells, lung cells, muscle cells, liver cells, fat cells, neuronal cells, glial cells, other brain cells, transformed cells of various cell lineages corresponding to normal cell counterparts (e.g. K562, HEL, HL60, and MEL cells), and established or otherwise transformed cells lines derived from all of the foregoing. In addition, the constructs of the present invention may be transferred by various means directly into tissues, where they would stably integrate into the cells comprising the tissues. Further, the constructs containing the DNA sequences of the peptides of the invention can be introduced into primary

cells at various stages of development, including the embryonic and fetal stages, so as to effect gene therapy at early stages of development.

- The described constructs may be administered in the form of a pharmaceutical preparation or composition containing a pharmaceutically acceptable carrier and a physiological excipient, in which preparation the vector may be a viral vector construct, or the like, to target the cells, tissues, or organs of the recipient organism of interest, including human and non-human mammals. The composition may be formed by dispersing the components in a suitable pharmaceutically acceptable liquid or solution such as sterile physiological saline or other injectable aqueous liquids. The amounts of the components to be used in such compositions may be routinely determined by those having skill in the art. The compositions may be administered by parenteral routes of injection, including subcutaneous, intravenous, intramuscular, and intrasternal.

- The following non-limiting examples illustrate various aspects and embodiments of the invention and should not be contrived as limiting the scope of the invention.

VI. EXAMPLES

The following materials were used in the examples described below.

- 20 Soluble IGF-1R was obtained from R&D Systems (Cat. # 391-GR/CF). Insulin receptor was prepared according to Bass *et al.*, 1996. The insulin is either from Sigma (Cat. # I-0259) or Boehringer. The IGF-1 is from PeproTech (Cat. # 100-11). All synthetic peptides were synthesized by Novo Nordisk, AnaSpec, Inc. (San Jose, CA), PeptioGenics (Livermore, CA), or Research Genetics (Huntsville, AL) at >80% purity. The Maxisorb Plates are from Nunc via Fisher (Cat. # 12565347). The HRP/Anti-M13 Conjugate is from Pharmacia (Cat. # 27-9421-01). The ABTS solution is from BioF/X (Cat. # ABTS-0100-04).

Example 1

A. Construction of Phage Library for Identifying IGF-1R and IR Binding Ligands

The schematic for the peptide library "RAPIDLIB™" on filamentous phage is shown in Figure 16. DNA fragments coding for peptides containing 40 random amino acids were generated in the following manner. A 145 base oligonucleotide was synthesized to contain the sequence (NNK)₄₀, where N = A, C, T, or G, and K = G or T. This oligonucleotide was used as the template in a PCR amplification along with two shorter oligonucleotide primers, both of which were biotinylated at their 5' ends. The resulting 190 bp product was purified and concentrated with QIAquick spin columns (QIAGEN, Inc. Valencia, CA), then digested with *Sfi*I and *Not*I. Streptavidin-agarose (GibcoBRL Life Technologies, Inc., Rockville, MD) was added to the digestion mixture to remove the cleaved ends of the PCR product as well as any uncut DNA. The resulting 150 bp fragment was again purified over QIAquick spin columns. The phagemid pCANTAB5E (Amersham Pharmacia Biotech, Inc., Piscataway, NJ) was digested with *Sfi*I and *Not*I, followed by phosphatase treatment. The digested DNA was purified using a 1% agarose gel followed by QIAEX II (QIAGEN). The vector and insert were ligated overnight at 15°C. The ligation product was purified using QIAquick spin columns (QIAGEN). Electroporations were performed at 1500 v in an electroporation cuvette (0.1 mm gap; 0.5 ml volume) containing 12.5 µg of DNA and 500 µl of TG1 electrocompetent cells (see below). Immediately after the pulse, 12.5 ml of pre-warmed (40°C) 2xYT medium containing 2% glucose (2xYT-G) was added and the transformants were grown at 37°C for 1 h. Cell transformants were pooled, the volume measured, and an aliquot was plated onto 2xYT-G containing 100 g/ml ampicillin (2xYT-AG) plates to determine the total number of transformants.

Sequence analysis of randomly selected clones indicated that 54% of all clones are in-frame (Mandecki *et al.*, 1997). The FLAG sequence (Hopp

et al., 1988) was incorporated into the library as an immunoaffinity tag as shown in Figure 16.

Another phage library expressing 20mer peptides, was constructed according to a similar procedure. The diversity of the library is 1.1×10^{11}
5 different clones.

B. Preparation of Electrocompetent Cells

To prepare electrocompetent cells, an overnight culture of *E. coli* TG1 cells (*F'*_{traD36 lacF⁺} $\Delta(lacZ)$ M15 *proAB*) / *supE* $\Delta(hsdM-mcrB)$ ₅ *r_K⁻m_K⁻* *McrB'*) *thi* $\Delta(lac-proAB)$ was diluted to an OD₆₀₀ = 0.05-0.1 in 500 ml 2xYT, then
10 grown at 37°C in 4 liter Ehrlenmyer flasks to an OD₆₀₀ = 0.5-0.6. The culture was poured into pre-chilled centrifuge bottles and incubated on ice for 30 min prior to centrifugation at 2000 x g for 30 min (2°C). The supernatant was poured off and the cell pellet was resuspended in a total of 400 ml of ice cold sterile distilled water. The process of centrifugation and resuspension
15 was repeated 2 times. After the last centrifugation, the pellet was resuspended in a total of 25 ml of ice cold water containing 10% glycerol. The cell suspension was transferred to pre-chilled 35 ml centrifuge bottles, and was then pelleted at 2000 x g for 10 min at 4°C. The cells were then suspended in 0.3 ml of the same 10% glycerol solution, aliquotted into
20 smaller tubes, and snap-frozen on dry ice. The aliquots were stored at -80°C.

To amplify the library, the transformants were inoculated into 4 l of 2xYT-AG medium and allowed to grow until the A₆₀₀ increased approximately 400 times. The cells were pelleted by centrifugation at 3000
25 x g for 20 min, then resuspended in 40 ml 2xYT-AG to which glycerol was added to a final concentration of 8%. The library was stored at -80°C.

C. Phage Rescue

This process was carried out using the standard phage preparation protocol with the following changes. Five individual recombinant cell
30 libraries, with a total diversity of 1.6×10^{10} , were combined and grown to

- OD₆₀₀ = 0.5 in 2xYT-AG at 30°C with shaking (250 rpm). Helper phage (M13K07) was then added (multiplicity of infection (MOI) = 15), and the cells were incubated for 30 min at 37° C without shaking, followed by 30 min at 37°C with shaking (250 rpm). Following infection, cells were pelleted and
- 5 the supernatant containing the helper phage was discarded. The cell pellet was resuspended in the initial culture volume of 2xYT-A (no glucose) containing 50 mg/ml kanamycin and grown overnight at 30°C with shaking (250 rpm). The cells from the overnight culture were pelleted at 3000 x g for 30 min at 4°C and the supernatant containing the phage was recovered.
- 10 The solution was adjusted to 4% PEG, 500 mM NaCl and chilled on ice for 1 h. The precipitated phage were pelleted by centrifugation at 10,000 x g for 30 min, then resuspended in phosphate-buffered saline (1/100 of the initial culture volume) and passed through a 0.45 µm filter. The phage were titred by infecting TG1 cells. The phage titer for the 40mer peptide library
- 15 was 4 x 10¹³ cfu/ml. The phage titer for the 20mer library was 3 x 10⁻³.

To amplify the library, the transformants were inoculated into 4 l of 2xYT-AG medium and allowed to grow until the OD₆₀₀ increased approximately 400 times. The cells were pelleted by centrifugation at 3000 x g for 20 min, then resuspended in 40 ml 2xYT-AG to which glycerol was

20 added to a final concentration of 8%. The library was stored at -80°C.

Example 2:

A. Panning IGF-1R

- A standard method was used to coat and block all microtiter plates. The soluble IGF-1R ("sIGF-1R") was diluted to 1 mg/ml in 50 mM sodium
- 25 carbonate buffer, pH 9.5. One hundred microliters of this solution was added to an appropriate number of wells in a 96-well microtiter plate (MaxiSorp plates, Nunc) and incubated overnight at 4°C. Wells were then blocked with MPBS (PBS buffer pH 7.5 containing 2% Carnation® non-fat dry milk) at room temperature (RT) for 1 h.

Eight wells were used for each round of panning. The phage were incubated with MPBS for 30 min at RT, then 100 μ l was added to each well. For the first round, the input phage titer was 4×10^{13} cfu/ml. For rounds 2 and 3, the input phage titer was approximately 10^{11} cfu/ml. Phage were
5 allowed to bind for 2 to 3 h at RT. The wells were then quickly washed 13 times with 200 μ l/well of MPBS. Bound phage were eluted by incubation with 100 μ l/well of 20 mM glycine-HCl, pH 2.2 for 30 s. The resulting solution was then neutralized with Tris-HCl, pH 8.0. Log phase TG1 cells were infected with the eluted phage, then plated onto two 24 cm x 24 cm
10 plates containing 2xYT-AG. The plates were incubated at 30°C overnight. The next morning, cells were removed by scraping and stored in 10% glycerol at -80°C. For subsequent rounds of affinity enrichment, cells from these frozen stocks were grown and phage were prepared as described above. A minimum of 72 clones were picked at random from the second,
15 third, and fourth rounds of panning and screened for binding activity. DNA sequencing of the clones revealed the abundance of sequences as summarized in Figure 18. Some of the clones (Figure 19) were frameshifted, that is, the relevant peptide sequence was encoded not in the FLAG frame, but in either frame + 1 or - 1.

20 B. ELISA Analyses of Phage

For phage pools, cells from frozen stocks were grown and phage were prepared as described above. For analysis of individual clones, colonies were picked and phage prepared as described above. Subsequent steps are the same for pooled and clonal phage. Microtiter wells were
25 coated and blocked as described above. Wells were coated with either IGF-1R or a control IgG mAb. Phage resuspended in MPBS were added to duplicate wells (100 μ l/well) and incubated at RT for 1 h. The phage solution was then removed, and the wells were washed 3 times with PBS at RT. Anti-M13 antibody conjugated to horseradish peroxidase (Pharmacia)
30 was diluted 1:3000 in MPBS and added to each well (100 μ l/well). Incubation was for 1 h at RT, followed by PBS washes as described. Color

was developed by addition of ABTS solution (100 μ l/well; Boehringer). Color development was stopped by adjusting each well to 0.5% SDS. Plates were analyzed at 405 nm using a SpectraMax 340 plate reader (Molecular Devices Corp., Sunnyvale CA) and SoftMax Pro software. Data points were averaged after subtraction of appropriate blanks. A clone was considered "positive" if the A_{405} of the well was ≥ 2 -fold over background.

For IC₅₀ determinations in a competitive ELISA, microtiter plates were coated with IGF-1R and blocked as described. Phage were prepared as described. Prior to addition of phage to plates, the peptide or recombinant variable antibody or fragment ("rVab"), or an appropriate control, was diluted in PBS and added to duplicate wells (100 μ l/well). After incubation for 1 h at RT, the prepared phage were added to each well (100 μ l/well) without removing the peptide or rVab solution. After incubation for 1 h at RT, the wells were washed and the color developed as described above.

The clones were next analyzed for binding to the receptor's active site (Figures 20A and 20B). Competitions of phage binding were done with the cognate ligand (i.e., IGF-1). All four phage clones tested, B6, F6, C6 and E5, bound to same site as IGF-1 since the binding of the clones to the immobilized IGF-1R could be inhibited with IGF-1.

To determine the rank order for phage peptides, the human IGF-1R (25 g/ml) was immobilized onto a CM-5 (BIAcore) sensor chip using amino coupling chemistry and the manufacturer's recommended protocol. The final surface density was 1000 RU. A monoclonal antibody was immobilized onto another flow cell as a control surface. Phage were directly injected (30-100 μ l) with a buffer flow rate of 1 μ l/min. Background binding to the control surface was subtracted prior to further analysis.

C. Phage Sequence Analysis

Sequence analysis of several clones shows that there are two distinct populations, designated as Class 1 (Formula motif 2) and Class II (Formula motif 1; Figure 21). Several of these have been chemically synthesized for subsequent testing. Class I peptides contain the consensus sequence D-x-

- F-Y-x-x-L-s-x-L, and are shown to be antagonistic in cell-based assays (Figure 22). Class II peptides contain the consensus N-F-Y-D-W-F-V, and are shown to be agonistic in cell-based assays (Figure 23). Neither of these consensus sequences have any significant linear sequence similarities greater than 2 or 3 amino acids with mature IGF-1.

Example 3: Assays with Synthetic Peptides

Four synthetic peptides, 5.1, 5.2, 5.3 and 5.4 (Figure 21) were made to study the properties of the artificial peptide ligands from phage display.

- Synthetic peptides were obtained from a commercial supplier (Anaspec). The peptides were supplied greater than 90% pure by HPLC. The molecular weights of the peptides as determined by mass spectroscopy agreed with the expected values.

- IGF-1R (100 $\mu\text{g/ml}$) was immobilized onto one flow-cell of a CM-5 sensor chip (Biosensor) using amine coupling chemistry and the manufacturer's recommended protocol. An unrelated IgG was immobilized in the same manner to another flow cell of the same chip as a control surface. Increasing concentrations of synthetic peptide were injected over both surfaces, and the binding responses were allowed to come to equilibrium. After subtraction of background binding from the control surface, the results were used to derive an equilibrium dissociation constant using Scatchard analysis (Figure 24A).

- In another experiment, IGF-1R (100 $\mu\text{g/ml}$) was immobilized onto a CM-5 sensor chip as described above, and an unrelated IgG was immobilized in the same manner to another flow cell of the same chip. IGF-1 alone, peptide 5.1 alone (corresponding to the B6 phage clone), or different mixes of the two, were injected over the derivatized chip surfaces. The results shown in Figure 24B indicate that the 5.1 peptide inhibits the binding of IGF-1, and the inhibition is increased by increasing amounts of the peptide. The results support the idea of an overlap of the peptide 5.1 binding site and the IGF-1 binding site on IGF-1R.

Example 4: Construction of Secondary Phage Libraries

Two phage libraries were designed on the basis of the sequences of the Class II binders known to possess agonistic properties in cell-based assays. The goal was to bring the affinity into a range that would allow the peptide to be used in a receptor binding assay and tested in a cell based assay for activity. Among several available mutagenesis methods, we chose one based on gene synthesis and phage display. In this method a library of doped oligonucleotides carrying several mutations in any single DNA molecule is used to obtain a pool of mutant genes, the expression products of which are phage displayed.

A. Phage Library A6L

The approach used was the doped synthesis of the oligonucleotide encoding the sequence of the peptide. The sequence encoding the peptide and the sequence of the synthetic oligonucleotide made are shown in Figures 25A-25B. The amino acid residues belonging to the consensus sequence were kept constant and were not mutated. The ratio of nucleosides in each condensation was chosen to provide an average of 6 nucleotide sequence changes at the DNA level and 4-5 mutations at the amino acid level over the length of the peptides. The regions corresponding to the FLAG, *SfiI* and *NotI* sites were not mutated.

The DNA sequence encoding the A6 peptide was optimized for *E. coli* codon usage by replacing a total of 24 nucleotides as shown in Figure 25A. The TAG stop codons (suppressed in the TG1 *E. coli* strain used) were replaced with CAG (glutamine). Then, the oligonucleotide sequence was designed to include doped nucleosides at positions corresponding to the coding region for the A6 peptide, except for the consensus NFYDWFV (Figure 25A). This synthetic oligonucleotide (Figure 25B) was then used as a template in a PCR reaction. The product of this PCR reaction was then purified, cut with *SfiI* and *NotI* restriction enzymes and cloned into the

I. Use of the Peptides Provided by this Invention

The IR and IGF-1R agonist and antagonist peptides provided by this invention are useful as potential therapeutics in pharmaceutical compositions, lead compounds for identifying other more potent or selective therapeutics, assay reagents for identifying other useful ligands by, for example, competition screening assays, and as research tools for further analysis of IR and IGF-1R. In particular, the peptide sequences provided by this invention can be used to design secondary peptide libraries, which include members that bind to Site 1 and/or Site 2 of IR or IGF-1R. Such libraries can be used to identify sequence variants that increase or modulate the binding and/or activity of the original peptide at IR or IGF-1R.

IR agonist amino acid sequences provided by this invention are useful as insulin analogs and may therefore be developed as treatments for diabetes or other diseases associated with a decreased response or production of insulin. For use as an insulin supplement or replacement, preferred amino acid sequence are: FHENFYDWFVRQVSK (D117, H2C),

DYKDFYDAIQLVRSARAGGTRDKK (D118, 20E2),
KDRAFYNGLRDLVGAVYGAWDKK (D119, 20C11),
DYKDLCQSWGVRIGWLAGLCPKK (D116, JBA5),
DYKDVTFTSAVFHENFYDWFVRQVSKK (D113, H2), and
GRVDWLQRNANFYDWFVAELG (S175). More preferred IR agonists are: FHENFYDWFVRQVSK (D117, H2C) and GRVDWLQRNANFYDWFVAELG (S175). Most preferred is GRVDWLQRNANFYDWFVAELG (S175). Preferred dimer sequences are represented by S170, S171, S172, S232, S300 sequences (see Table 15).

IGF-1R antagonist amino acid sequences provided by this invention are useful as treatments for cancers, including, but not limited to, breast and prostate cancers. Human and breast cancers are responsible for over 40,000 deaths per year, as present treatments such as surgery, chemotherapy, radiation therapy, and immunotherapy show limited success. The IGF-1R antagonist amino acid sequences disclosed herein are also

useful for the treatment or prevention of diabetic retinopathy. Recent reports have shown that a previously identified IGF-1R antagonist can suppress retinal neovascularization, which causes diabetic retinopathy (Smith *et al.*, 1999).

- 5 IGF-1R agonist amino acid sequences provided by this invention are useful for development as treatments for neurological disorders, including stroke and diabetic neuropathy. Reports of several different groups implicate IGF-1R in the reduction of global brain ischemia, and support the use of IGF-1 for the treatment of diabetic neuropathy (reviewed in Auer *et al.*, 1998; Apfel, 1999).
- 10

J. Methods of Administration

- The amino acid sequences of this invention may be administered as pharmaceutical compositions comprising standard carriers known in the art for delivering proteins and peptides and by gene therapy. Due to the labile
- 15 nature of the amino acid sequences parenteral administration is preferred. Preferred modes of administration include aerosols for nasal or bronchial absorption; suspensions for intravenous, intramuscular, intrasternal or subcutaneous, injection; and compounds for oral administration. Other modes of administration and examples of suitable formulative components
- 20 for use with this embodiment are discussed below. Other modes of administration include intranasal, intrathecal, intracutaneous, percutaneous, enteral, and sublingual. For injectable administration, the composition is in sterile solution or suspension or may be emulsified in pharmaceutically- and physiologically-acceptable aqueous or oleaginous vehicles, which may
- 25 contain preservatives, stabilizers, and material for rendering the solution or suspension isotonic with body fluids (i.e. blood) of the recipient. Excipients suitable for use are water, phosphate buffered saline, pH 7.4, 0.15 M aqueous sodium chloride solution, dextrose, glycerol, dilute ethanol, and the like, and mixtures thereof. Illustrative stabilizers are polyethylene glycol,
- 30 proteins, saccharides, amino acids, inorganic acids, and organic acids, which may be used either on their own or as admixtures. The amounts or

quantities, as well as routes of administration, used are determined on an individual basis, and correspond to the amounts used in similar types of applications or indications known to those of skill in the art.

The constructs as described herein may also be used in gene transfer and gene therapy methods to allow the expression of one or more amino acid sequences of the present invention. Using the amino acid sequences of the present invention for gene therapy may provide an alternative method of treating diabetes which does not rely on the administration or expression of insulin. Expressing insulin for use in gene therapy requires the expression of a precursor product, which must then undergo processing including cleavage and disulfide bond formation to form the active product. The amino acid sequences of this invention, which possess activity, are relatively small, and thus do not require the complex processing steps to become active. Accordingly, these sequences provide a more suitable product for gene therapy.

Gene transfer systems known in the art may be useful in the practice of the invention. Both viral and non-viral methods are suitable. Examples of such transfer systems include, but are not limited to, delivery via liposomes or via viruses, such as adeno-associated or vaccinia virus. Numerous viruses have been used as gene transfer vectors, including papovaviruses (e.g., SV40, adenovirus, vaccinia virus, adeno-associated virus, herpes viruses, including HSV and EBV, and retroviruses of avian, murine, and human origin). As is appreciated by those in the art, most human gene therapy protocols have been based on disabled murine retroviruses. Recombinant retroviral DNA can also be employed with amphotrophic packaging cell lines capable of producing high titer stocks of helper-free recombinant retroviruses (e.g., Cone and Mulligan, 1984).

A recombinant retroviral vector may contain the following parts: an intact 5' LTR from an appropriate retrovirus, such as MMTV, followed by DNA containing the retroviral packaging signal sequence; the insulator element placed between an enhancer and the promoter of a transcription

unit containing the gene to be introduced into a specific cell for replacement gene therapy; a selectable gene as described below; and a 3' LTR which contains a deletion in the viral enhancer region, or deletions in both the viral enhancer and promoter regions. The selectable gene may or may not have a 5' promoter that is active in the packaging cell line, as well as in the transfected cell.

The recombinant retroviral vector DNA can be transfected into the amphotrophic packaging cell line Ψ -AM (see Cone and Mulligan, 1984) or other packaging cell lines which are capable of producing high titer stocks of helper-free recombinant retroviruses. After transfection, the packaging cell line is selected for resistance to G418, present at appropriate concentration in the growth medium. Adenoviral vectors (e.g. DNA virus vectors), particularly replication-defective adenovirus vectors, or adeno-associated vectors, have been described in the art (Kochanek *et al.*, 1996; Ascadi *et al.*, 1994; Ali *et al.*, 1994).

Nonviral gene transfer methods known in the art include chemical techniques, such as calcium phosphate co-precipitation, direct DNA uptake and receptor-mediated DNA transfer, and mechanical means, such as microinjection and membrane fusion-mediated liposomal transfer. In addition, viral-mediated gene transfer can be combined with direct *in vivo* gene transfer using liposomes, thereby allowing the delivery of the viral vectors to tumor cells, for example, and not to surrounding non-proliferating cells. A description of various liposomes which are stated as being useful for transferring DNA or RNA into cells is present in United States Patents 5,283,185 and 5,795,587. The retroviral vector producer cell line can also be injected directly into specific cell types, e.g., tumors, to provide a continuous source of viral particles, such as has been approved for use in patients afflicted with inoperable brain tumors.

Receptor-mediated gene transfer methods allow targeting of the DNA in the construct directly to particular tissues. This is accomplished by the conjugation of DNA (frequently in the form of a covalently closed supercoiled

pCANTAB5E vector as described for the original peptide library. Over 10^{10} different clones were obtained in the final library.

B. Phage Library A6S

- While the consensus sequence NFYDWFV was kept constant in the
- 5 A6S library, the flanking regions were randomized in the A6S library as shown in Figure 26A. The codons in the random region were of the NNK type to reduce the frequency of stop codons (N = A, C, G, or T; K = G or T). The sequence of the synthetic oligonucleotide made is given in Figure 26B. This synthetic oligonucleotide was then used as a template in a PCR
- 10 reaction. The product of this PCR reaction was then purified, cut with *Sfi* I and *Nof* I restriction enzymes and cloned into the pCANTAB5E vector as described for the original peptide library. Over 10^9 different clones were obtained in the final library.

C. Secondary Phage Library Based on Clone H5

- 15 Peptide H5 (LCQRLGVGWPGWLSGWCA) was identified in an independent experiment as a binder to the rat growth hormone binding protein. This peptide and four other H5-like peptides, including 2C3-60 (Figure 27), were found in cell culture experiments to possess agonistic activity toward IGF-1R⁺ cells, but not against IGF-1R⁻ cells. Further,
- 20 subsequent *in vitro* experiments showed that the H5-like peptides are not competed by IGF. This suggests that these peptides recognize a second allosteric site on IGF-1R. BIAcore analysis showed that binding of the 2C3-60 peptide to IGF-1R is ~ 20 μ M. Subsequently, a phage library of mutants of the H5 sequence was constructed and used for panning against IGF-1R.
- 25 Gene synthesis to introduce mutations and phage display were used to construct an H5 secondary library. In this method a library of doped oligonucleotides carrying several mutations in any single DNA molecule is used to obtain a pool of mutant genes which are phage displayed. This method allowed the encoding of both the original H5 peptide as control as

well as versions containing high numbers of mutations per peptide in a very large library ($>10^{10}$).

- Therefore, the H5 secondary mutant library was designed to contain an average of four amino acid changes (mutations) per peptide. The number of possible mutant H5 peptide sequences having four mutations is 1.0×10^{10} and is equivalent to the actual size of the secondary phage library. Sequence analysis indicates that of these peptides 30% have 3-4, 33% have 1-2 and 32 % have 5-6 mutations. There also was a small percent with 7-8 mutations and 5% clones without any mutation.

- 10 An oligonucleotide based on the DNA sequence encoding the H5 peptide was synthesized. The sequence of the oligonucleotide is:
5'-CTACAAAGACCTGTGTTAGAGTTTGGGGGTACGTATCCGGGTGGT
TGGCGGGGTGGTGTGCGGCGGCCGCAGTGTGA-3'

- 15 The underlined base positions were synthesized as mixtures of four nucleosides as follows:

A = 90% A; 3.3% C; 3.3% G; and 3.3% T

C = 3.3% C; 90% C; 3.3% G; and 3.3% T

G = 3.3% C; 3.3% C; 90% G; and 3.3% T

T = 3.3% C; 3.3% C; 3.3% G; and 90% T

- 20 Using this oligonucleotide as a template, the H5 secondary library was constructed, electroporated, amplified, and rescued essentially as described for the original peptide library. The final diversity of this secondary library was $\sim 10^{10}$.

D. Characterization of Libraries

- 25 Forty-eight randomly picked clones from each of the secondary libraries (Round 0, before panning) were rescued and the phage was assayed in an ELISA for binding to the anti-E-tag mAb, as well as for binding to IGF-1R (E-tag is used as an indicator of expression of displayed peptides on phage surfaces). The results showed that although most of the clones in

- the two libraries (70%) display a peptide (i.e., are positive for E-tag), only about 6% of the clones from the A6 long (A6L) library bind to IGF-1R by phage ELISA, and none of the 24 clones tested from the A6 short (A6S) library bind to IGF-1R. This indicates that the most common outcome of random mutagenesis is the loss of IGF-1R affinity. Nevertheless, some mutants do retain their binding properties and some have improved affinities (see below).

E. Panning with the Secondary Libraries

- The two secondary libraries of Example 4 were used in a panning experiment against IGF-1R. Approximately 50 clones from each four rounds of panning were analyzed in a phage ELISA to identify the clones that bind to the receptor. The positive clones were subjected to DNA sequencing and protein sequence comparison. Figure 28 provides a listing of different sequences obtained from panning with the A6S library. The results show that a variety of phage peptide sequences can bind to IGF-1R, while the consensus sequence NFYDWFV is preserved in the majority of instances.

The H5 secondary phage library was panned against IGF-1R to find H5-like peptides with higher affinities for IGF-1R

- The H5 Library has a diversity of $\sim 2.6 \times 10^{10}$ clones with a phage titer of 1.0×10^{13} phage ml^{-1} . A total of three rounds of panning were performed. Table 2 summarizes the results from the three rounds of panning and shows the ELISA results for the individual clones selected from each round, the number of clones examined in each round of panning, as well as the number and percentage of E-Tag⁺ clones and IGF-1R⁺ clones.

F. **TABLE 2:** Results of panning with the H5 secondary phage library.

Round	Total	E-Tag ^a		IGF-1R ^b	
		Number	%	Number	% Total
0	32	22	69 %	0	0 %
1	128	116	91 %	1	1 %
2	128	108	84 %	2	2 %
3	160	116	91 %	65	51 %

^aE-Tag⁺ means ELISA absorbance values >2X background. ^bIGF-1R⁺ means ELISA Absorbance >2X background. Background absorbance values are 0.05 to 0.075.

5

Each of the IGF-1R⁺ clones were sequenced, as were 15 IGF-1R⁻ clones with high E-Tag values (Absorbance >1.0). These sequences are shown in Figure 29. There is no discernible difference between binding sequences and the non-binding sequences with the exception that all of the binding sequences hold the Gly at position 6 constant. All sequences, binding and non-binding, hold the TAG stop codon constant at position 3 (the *E. coli* strain used in phage production contains the *supE44* mutation, therefore Gln replaces the TAG and it denoted in Figure 29 by Q). This suggests TAG stop codon is required for phage production and not binding.

10

15 **Example 5: Construction of the rVab
Recombinant Antibody Variable Region library**

The design, expression and purification of single-chain antibodies has been reviewed (Rader and Barbas, 1997; Hoogenboom, 1997). Briefly, the variable portion of the heavy chain (V_H) is linked to the variable portion of the light chain (V_L) by a flexible peptide linker. Random combinations of V_H and V_L genes can be genetically combined to provide some of the diversity required for a library of recombinant variable region antibodies (rVabs) (Figure 30). In our library, further diversity is provided by full randomization

20

of the 6-12 amino acids comprising the V_H CDR3 (indicated as "D" in Figure 30).

A total of 49 human genomic v_H genes and ten human genomic v_L genes (Figure 31) were isolated from total human genomic DNA by PCR.

- 5 The other genetic components of the library (V_H, CDR3, j_H, linker, and j_L) were derived from synthetic oligonucleotides. Assembly of these components was done using directional cloning as outlined in Figure 32 and Figure 33.

A. Ligations

- 10 The general schematic for the assembly of the rVab library ("GRABLIBTM") is provided in Figure 30. Four gene fragments (V_H, VHCDR3/JH/LINKER, V_L and J_L) were ligated together in the proper orientation and cloned into pCANTAB 5E (Pharmacia). Directional cloning was achieved using the *BsrDI* restriction enzyme (Figure 32). Forty-nine
- 15 germline V_H segments and ten V_L segments encoding many of the genes from the human V_H and V_L repertoire were isolated (Figure 31) using the polymerase chain reaction. V_H CDR3 (ranging from 6 to 12 amino acids) /JH/Linker fragments were generated by ligation of four oligonucleotides (WM 2.1, 2.2, 2.3 and 2.4) and cloning the resulting fragment into the
- 20 plasmid pUC18 previously cut with *KpnI* and *HindIII*. The insert was then amplified using PCR and oligonucleotide primers to introduce a synthetic D-segment of 6 to 12 amino acids having a random sequence and the *BsrDI* restriction site. The J_L gene fragments were assembled as a result of annealing of two synthetic oligonucleotides. The assembled fragments (200
- 25 ng) were used as template in a PCR amplification along with two shorter oligonucleotide primers, both of which were biotinylated at their 5' ends. The resulting 800 bp product was purified and concentrated with QIAquick spin columns (QIAGEN), then digested with the *SfiI* and *NotI* restriction enzymes. Streptavidin-agarose (GibcoBRL) was added to the digestion
- 30 mixture to remove the cleaved ends of the PCR product as well as any

uncut DNA. The resulting 800 bp fragment was purified by passing DNA over QIAquick spin columns.

- Phagemid pCANTAB 5E (Pharmacia) was digested with the *Sfi*I and *Nof*I restriction enzymes, which was followed by the alkaline phosphatase treatment to dephosphorylate the ends of the restriction fragments generated. The digested DNA was purified by running the digested plasmid DNA on a 1% agarose gel, followed by the DNA purification using the QIAEX II (QIAGEN) column. The vector and insert DNA were ligated overnight at 16°C. The ligation product was purified using QIAquick spin columns (QIAGEN) and electroporations were performed at 1500 v in a electroporation cuvette (0.1 mm gap; 0.5 ml volume, BTX, Inc.). The amount of DNA in one electroporation was 12.5 µg per 500 µl of TG1 electrocompetent cells. Immediately after the pulse, 12.5 ml of a pre-warmed (40°C) 2xYT medium containing 2% glucose (2xYT-G) was added, and the transformants were grown at 37°C for 1 h. The transformants were pooled, the volume measured, and an aliquot was plated onto the 2xYT-G medium containing 100 µg/ml ampicillin (2xYT-AG) plates to determine the total number of transformants. The number of different transformants and the diversity of the library was 3×10^{10} .
- The electrocompetent cell preparation, phage library amplification, library phage rescue, phage preparations and coating of microtiter plates were done as described above for the peptide library.

B. Panning for IGF-1R Binders with rVab Antibody Library

1. Panning Procedure

- Panning of the antibody library was done essentially as described for the peptide library, for a total of four rounds. Of the 200 clones tested, approximately 10% bound specifically to sIGF-1R. Among these specific binders, 40% can be competed by IGF-1 for receptor binding. The clonal analysis and DNA sequencing (Figures 31-39) followed by ELISA and cell-based assays (Figures 40-46) have shown that two clones, 43G7 and M100,

are agonistic with ED_{50} values of approximately 20 nM (a plot for the 43G7 antibody is shown in Figure 41). Two other rVabs, 1G2P and 39F7, have been shown to be antagonistic, with IC_{50} values of approximately 20 nM (Figure 42).

- 5 Microtiter wells were coated with IGF-1R as described above, with eight wells being used for each round of panning. The phage were incubated with MPBS for 30 min at RT, then 100 μ l of the phage suspension was added to each well. For the first round, the input phage titer was 8×10^{13} cfu/ml. For rounds 2 and 3, the input phage titer was approximately
- 10 10^{11} cfu/ml. Phage were allowed to bind for 2 to 3 h at RT. The wells were then quickly washed 13 times with 200 μ l /well of MPBS. Bound phage were eluted by incubation with 100 μ l/well of 20 mM glycine-HCl, pH 2.2 for 30 s. The resulting solution was then neutralized with Tris-HCl, pH 8.0. Log phase TG1 cells were infected with the eluted phage, then plated onto two 4
- 15 cm x 4 cm plates containing 2XYT-AG. The plates were incubated at 30°C overnight. The next morning, cells were removed by scraping and stored in 10% glycerol at -80°C. For subsequent rounds of affinity enrichment, cells from these frozen stocks were grown and phage were prepared as described above.

20 2. Elisa Analyses Of Phage Pools

- To prepare the phage pools, cells from frozen stocks were grown and phage were prepared as described above. Microtiter wells were coated and blocked as described above. The wells were coated with either IGF-1R (R&D Systems, Inc.) or with control BSA. Phage resuspended in MPBS
- 25 were added to duplicate wells (100 μ l/well) and incubated at RT for 1 h. The phage solution was then removed, and the wells were washed 3 times with PBS at RT. Anti-M13 antibody conjugated to horseradish peroxidase (Pharmacia) was diluted 1:3000 in MPBS and added to each well (100 μ l/well). Incubation was for 1 h at RT, followed by PBS washes as
- 30 described. Color was developed by addition of ABTS solution (100 μ l/well;

- Boehringer). Color development was stopped by adjusting each well to 0.5% SDS. Plates were analyzed at 405 nm using a SpectraMax 340 plate reader (Molecular Devices) and SoftMax Pro software. Data points were averaged after a subtraction of appropriate blanks. Phage pools was considered "positive" if the A_{405} of the well was > 2-fold over background.

3. Competition ELISAs

- For IC_{50} determinations, microtiter plates were coated with IGF-1R and blocked as described. Phage and soluble rVabs were prepared as described above. Prior to addition of phage or soluble rVabs to the plates,
- 10 IGF-1 solution in PBS (1 μ g/ml) was added to duplicate wells (100 μ l/well). After incubation for 1 h at RT, the prepared phage were added to each well (100 μ l/well) without removing the IGF-1 solution. After incubation for 1 h at RT, the wells were washed and the color was developed as described above.
- 15 Six rVab clones bound specifically to IGF-1R. The sequences of the clones are shown in Figure 34-39.

4. Expression And Purification Of Soluble rVabs

- E. coli* HB2151 carrying the rVab genes on the pCANTAB5E plasmid
- 20 (Pharmacia) were grown in 2xYT supplemented with 100 μ g/ml ampicillin and 1% glucose at 37° C overnight and then subcultured in the absence of glucose at an OD_{600} of 0.1, and grown at 21° C until OD_{600} was 1.0. Expression was induced by the addition of IPTG to 1 mM and the cells were grown for 16 h at 30° C. The cells and culture supernatant were separated
- 25 by centrifugation and samples of the cell pellet and supernatant were analyzed on a 15% SDS-PAGE gel followed by the Western blot analysis using the mouse monoclonal antibody anti-E-Tag-HRP conjugate (Pharmacia) to visualize the expressed product. The expressed rVabs were purified from the supernatant by precipitation with ammonium sulphate
- 30 (which was added to 70% saturation) at 21° C, followed by centrifugation at

10,000 g for 15 min. The aqueous phase was discarded, and the pellet resuspended and dialyzed in PBS (phosphate buffered saline, pH 7.4) at 4° C overnight. Insoluble material was removed by centrifugation at 10,000 g, and the supernatant was filtered through a 0.22 µm membrane and purified on an anti-E-Tag antibody affinity column (Pharmacia). The affinity resin was equilibrated in TBS (0.025 M Tris-buffered saline, pH 7.4) and the bound protein was eluted with the Elution buffer (100 mM glycine, pH 3.0). The rVab was concentrated to 1 mg/ml, dialyzed against TBS and stored at 4° C. The SDS-PAGE, Western blot analysis and N-terminal sequence analysis of the affinity purified material were performed according to standard protocols.

5. Size Exclusion FPLC Chromatography

The affinity purified rVabs were fractionated by size exclusion FPLC on a Superdex 75 HR10/30 column (Pharmacia) to determine the molecular size and aggregation state of the rVabs. For calibration of the column, High and Low Molecular Weight Gel Filtration Calibration Kits (Pharmacia) were used. Fractions from several chromatographic separations corresponding to a molecular weight of 30 kDa were pooled and concentrated to 0.7-1.0 mg/ml using Amicon XM10 membranes. Protein concentrations were determined using the BCA protein assay kit (Pierce Chemical Co., Rockford, IL).

6. BIACore Analyses

IGF-1R was immobilized onto one flow cell of a CM-5 sensor chip (Biosensor) using amine coupling chemistry and the manufacturer's recommended protocols. BSA was immobilized in the same manner to another flow cell of the same chip as a control surface. Increasing concentrations of the affinity-purified rVabs were injected over both surfaces, and the binding responses were allowed to come to equilibrium. After a subtraction of the background binding (from the control surface), the equilibrium dissociation constant was derived using Scatchard analysis.

7. Time-Resolved Fluorescence Assay

We have selected the basic format of an *in vitro* competitive receptor binding assay as the basis of a heterogeneous screen for small organic molecular replacements for IGF-1. In the present assay, occupation of the active site of IGF-1 receptor is quantified by time-resolved fluorometric detection (TRFD) with streptavidin-labeled europium (saEu) complexed to biotinylated peptides (bP). In this assay, saEu forms a ternary complex with bP and IGF-1 receptor (i.e., IGF-1R:bP:saEu complex). The TRFD assay format is well established, sensitive, and quantitative (Tompkins *et al.*, 1993). We demonstrate the assay using 43G7 rVab or a biotinylated peptide. Furthermore, we show that both assay formats faithfully report the competition of the biotinylated ligands binding to the active site of IGF-1R by IGF-1.

In these assays, soluble IGF-1 receptor is coated on the surface of microtiter wells, blocked by PBS containing milk and BSA, and then incubated with biotinylated peptide or rVab. Unbound bP is then washed away and saEu is added to complex with receptor-bound bP. Upon addition of the acidic enhancement solution, the bound europium is released as free Eu^{+3} which rapidly forms a highly fluorescent and stable complex with components of the enhancement solution. The IGF-1R:bP bound saEu is then converted into its highly fluorescent state and detected by TRFD.

a. Preparation of [Eu³⁺]-Labeled rVab 43G7

One milligram of rVab 43G7 (the sequence is provided in Figure 34) was added to 300 nmol Eu^{3+} -chelated $\text{N}^1(\text{P-isothiocyanatobenzyl})$ -diethylenetriamine- $\text{N}^1, \text{N}^2, \text{N}^3$ -tetracetic acid (Wallac). The reaction was conducted at pH 8.5. The tube was mixed gently and placed at ambient temperature. When the reaction was complete (16 h), the sample was diluted 10-fold into the Tris-buffered saline (TBS), pH 7.5, and the separation of the labeled rVab from the unlabeled rVab and free- Eu^{3+} was achieved by using the PD-10 column. The protein concentration and

labeling efficiency were determined using a Europium standard solution (Wallac).

b. Assay Method

IGF-1R (5 µg/ml in 50 mM NaHCO₃) was coated onto low-fluorescence MaxiSorp (Nunc) plates (100 µl/well) overnight at 4°C. The plates were blocked with PBS containing 2% non-fat milk and 0.05% BSA for 2 h at RT, followed by three PBS washes. For competitive ELISA, serial dilutions of unlabelled IGF-1 (0.1 nM-100 µM) were added to the plates (100 µl/well) and incubated at RT for 1-2 h. 100 µl [Eu³⁺] rVab 43G7 in Wallac's DELFIA assay buffer (100 mM Tris-HCl, pH 7.8; 150 mM NaCl; 0.5% BSA, 0.05% bovine Ig; 0.05% NaN₃; 0.01% Tween-20) was added and incubated for 1.5 h at RT. The plates were then washed 5 times with TTBS (TBS buffer containing Tween-20; Wallac) and tapped dry. Subsequently, 100 µl of DELFIA enhancement solution (100 mM acetone-potassium hydrogen phthalate, pH 3.2; 15 mM 2-naphthyltrifluoroacetate; 50 mM tri(n-octyl)-phosphine oxide; 0.1% Triton X-100) was added to each well, and the plates were shaken for 10 min at RT. Fluorescence of each sample well was measured at 615 nm using a DELFIA 1234 fluorometer (EG&G Wallac).

The dose response of TRFD of Eu was studied in microtiter wells. Detection is linear over the range 0.2 to 200 fmol with a limit of detection (twice background) of 0.05 fmol. There are 6010 fluorescent units (FU) per fmol of Eu. Binding and detection of Eu-SA, (4.7 mol Eu/mol streptavidin) to wells coated with biotinylated BSA (bBSA) (6 mol biotin/mol BSA) is linear over the entire range tested. The specific fluorescent activity of streptavidin Eu-SA (with 4.7 mol Eu/mol SA) is 28 kfu/fmol and the limits of detection (i.e., twice background) are 0.030 fmol. Coating with IGF-1R was linear up to inputs of 200 ng/well and thereafter appeared to saturate at about 660 ng bIGF-1 (biotinylated IGF-1) per well. This is the expected amount based on the manufacturer's information about protein saturation densities of these wells (Nunc manual). These studies show a limit of detection of bIGF-1 (i.e.,

twice background) of 0.05 fmol bIGF-1. The ability of this assay format to detect specifically bound bIGF-1 (or bPeptides) to IGF-1R coated wells was determined.

8. Elisa Analyses

5 ELISA was performed on selected rVabs. We found that the native IGF-1 ligand inhibits the binding of peptide 5.1 (the sequence of which originates from the phage clone B6) as shown in Figure 43. The detection of the peptide involved a sandwich configuration with the Eu-labeled streptavidin. It was determined that the binding of Eu-labeled rVab 43G7 to
10 IGF-1R is inhibited by IGF-1 with an IC_{50} of approximately 2 nM, as shown in Figure 44. The binding of the biotinylated peptide 5.1 is inhibited by rVab 43G7 with an IC_{50} of about 10 nM (Figure 45), indicating that both the peptide and rVab bind to the same site on the IGF-1R molecule.

Figures 46A-46D demonstrates the binding properties of the 43G7
15 antibody. The binding of the Eu-labeled 43G7 antibody is competed by peptide 5.1 (clone B6) (Figure 46A) and by the non-labeled 43G7 (Figure 46B), as well as by rVab 39F7 (Figure 46C) and rVab 1G2P (Figure 46D). The sequences of rVabs 1G2P and 39F7 are provided in Figure 35 and Figure 36, respectively.

20 C. **Conclusions**

The above results support the use of this assay procedure as a high throughput screen for agents, with affinities for sites on the human IGF-1R which bind IGF-1. The studies show the IGF-1-specific peptides bind in a dose-dependent, saturable manner and are blocked from binding by agents
25 known to bind to the active site of the receptor. This competition is reproducible and easily quantified. Furthermore, the TRFD assay, which is automatable, is much more sensitive than is an ELISA.

Example 6: Agonistic and Antagonistic Activity of IGF-1R-Binding Peptides

Agonistic and antagonistic activities of the IGF-1-specific peptides were tested in FDCP2 cells (NIH) which express IGF-1R. The cell line requires either IL-3 or IGF-1 for growth, and the cells were maintained in RPMI 1640 medium containing 15% FCS (fetal calf serum). Agonism activity assays were performed in a total volume of 100 μ l in 96 well plates (flat bottom). Cells were seeded at 30,000 cells/well in 50 μ l RPMI 1640 (without IL-3) medium containing 15% FCS in triplicate wells. To each well, 50 μ l of a solution containing either IGF-1, rVabs or peptides at different concentrations was added, followed by incubation for 42 h in a CO₂ incubator at 37°C.

Assays to measure the antagonistic activity were performed in a total volume of 100 μ l in 96 well plates. An IGF-1-specific peptide, rVab or an appropriate control was added to wells containing 0.003 μ M of human IGF-1 and incubated at 37°C for 18 h in CO₂ incubator. Proliferation assays were performed using WST-1 reagent. The WST-1 tetrazolium salt (slightly red) is cleaved to formazan (dark red) by the succinate-tetrazolium reductase system, which is active only in viable cells. An increase in the number of cells results in an increase in the overall activity of the dehydrogenase which results in a higher absorbance at 450 nm. Ten microliters of WST-1 reagent was added to each well and the plates incubated for 1-4 h at 37°C. Proliferation was measured by absorbance at 450 nm. Both 5.3 and 5.4 peptides showed an agonistic activity at the 10 μ M concentration (Figure 23). Peptides 5.1 and 5.2 showed a significant antagonistic activity in the 3-30 μ M concentration range (Figure 22). Control peptide showed no antagonistic activity at the concentrations tested.

The results described demonstrate the feasibility of both the chemical synthesis of and construction of a recombinant expression vector to make sufficient soluble peptide (free or as fusion with some carrier protein) or rVab for testing agonist and antagonist activities. The results provide peptide-

receptor pairs to be used in a site directed competition binding assay wherein IGF-1R can be used as one member of the pair, with the peptide or a rVab as the other member. Labeling of each member, and detection of pair formation, using either member in radioactive or nonradioactive labeled forms, is possible by a variety of methods known to those skilled in the art of building competition binding assays. This assay provides a high throughput screening assay to identify small organic molecules which bind to the active site of IGF-1R.

Example 7: Phage Library B6-2

10 This library was designed based on the "core" sequence of the Class I binders Site 1(B6) which posses antagonistic activities in a cell proliferation assay. The core sequence was determined as DPFYHKLSEL, where the residues F (position 3, X₆ of Formula 2), Y (position 4, corresponding to X₇ of Formula 2), L (position 7, corresponding to X₁₀ of Formula 2) and L (position 10, corresponding to X₁₃ of Formula 2) were the only residues observed at those positions. The purpose of this library was to test the possibility that some binders will show deviations from the core sequence, especially at the positions where substitutions had not previously been observed. The library was therefore made from doped oligonucleotides so that, on average, half of 15 the amino acid residues were altered per peptide. The library was made as described in the original B6 library, i.e., synthetic oligonucleotides were first amplified in a PCR reaction. The resultant products were cloned into pACANTAB5E (Pharmacia) via *Sfi*I and *Not*I restriction sites as previously described. Over 10¹⁰ different clones were obtained in the final library.

A. Random 20mer Library

1. Panning with the B6-2 and Random 20mer Libraries

The libraries were affinity selected against IGF-1R. 96 clones from round 3 of panning from B6-2 library and 96 clones from round 4 from the

random 20mer library were analyzed in a phage ELISA to identify binders. The DNA of binders was then determined. The results from both libraries show that positions other than positions 3, 4, 7 and 10 as described above can vary relatively at ease (see tables below), while variability at positions 3, 4, 7 and 10 is much more restricted. The results from the B6-2 library show that the restricted core residues were maintained in all binders except one, which happened only in one instance, L (position 7) can be substituted by another hydrophobic residue, M, at that position. The result from the random 20 library panning revealed that another aliphatic amino acid residue, I can substitute for L at position 7. In addition, the restricted residue at position 10 (L) can also be substituted with amino acid residue M. Thus, 2 of the previously identified restricted residues (L at positions 7 and 10) are not absolute, even though L is preferred at these positions. It should be noted that the failure to observe a substitution at a particular residue position does not necessarily indicate that substitutions cannot be made without losing activity, rather such an absence of substitution is indicative of a preference or an aversion for substitution. The findings are summarized below:

B. Results

Combined results from binding clones isolated from B6-2 (doped core) and random 20 libraries of the Formula 2 motif are shown below in Table 3. Sequences from 25 clones from B6-2 and 29 clones from the random 20mer library were analyzed. Numbers adjacent the amino acid residues represent the frequency with which a specific amino acid was observed at the corresponding position.

TABLE 3

						*	*		*			*	
	B6	CORE	D37	P34	F54	Y54	H12	K15	L46	S16	E27	L53	L30
5			E	A			A4	A10	I7	A5	A6	M	A6
			G3	D4			D7	G7	M	D3	D3		I
			K4	E			E9	I		E4	G2		K
			R2	G10			G	L8		F5	K		S2
10			S5	L			K3	M3		G4	L		T
			T	Q			L2	N		H	Q3		V13
			V	S			M	Q		L6	R6		
				T			N	R5		M	S4		
15							Q7	T		N	V		
							R4	V		Q2			
							S	W		R2			
							T			T2			
							V			Y2			

Based on the substitutions observed above, the following preferences shown in Table 4 are preferred for substitutions in the amino acid sequence of Formula 2 for binding to IGF-1R.

TABLE 4

25X98	X99	X6	X7	X8	X9	X10	X11	X12	X13	X100
1(D)	2(P)	3(F)	4(Y)	5(H)	6(K)	7(L)	8(S)	9(E)	10(L)	11(L)
no aromatics; no large aliphatics; no c	no aromatics; no c; no + charged			no aromatics; no C; no P; no I	no aromatics, except W; no - charged; no C; no P		no C; no P	no aromatics; no C; no P		no aromatics; no aliphatics; no C; no P

Example 8:

A composite of amino acid residues observed in sequences of random 20mer, 40mer and A6 (Formula 1) clones is illustrated below:

	A6 CORE	N	F	Y	D	W	F
5		D6				A	
		E				E9	
		G6				G2	
		H3				Q4	
10		K				R	
		P				S	
		Q8					
		S					
		T					
15		V					

A summary of preferences for A6 residues is shown in Table 5 below.

An illustration of residues which are characteristic of IGF-1R binding sequences (above parental sequence) and those which are not typically associated with binding sequences (below parental sequence). Table 6.

TABLE 5

	X1	X2	X3	X4	X5
1(N)	2(F)	3(Y)	4(D)	5(W)	6(F)
no aromatics; no large aliphatics; no C; no P			no hydrophobics, except tiny; no C; no P		

																	M
5																	K
										Y							R
										G							S
										T							D
										K							L
10										s	A						A
										H	G						E
										Q	Q						Q
									D	D Y E							V A Q V T
Parental	D	Y	K	D	G	F	R	E	G	N F Y D W F V A P							P
15										T Q Q A R L L							
										V L F E G V E							
										E V L G P W M							
										G * E R Q C D							
										A Y S H C							
Uncharacteristic of IGF-1R Binding Sequences										Q S I S							
20										I N *							
										Y K							
										K	*						

A standard method was used to coat and block all microtiter plates. IR (prepared according to Bass *et al.*, 1996) was diluted to 2 µg/ml in PBS. Fifty microliters of this solution was added to an appropriate number of wells in a 96-well microtiter plate (MaxiSorp plates, Nunc) and incubated overnight at 4°C. Wells were then blocked with a solution of 2% non-fat milk in PBS (MPBS) at RT for at least 1 h.

Eight wells coated with IR were used for each round of panning. One hundred microliters of phage were added to each well. For the first panning
35 round, the input phage titer was 4×10^{13} cfu/ml. For subsequent rounds, the

input phage titer was approximately 10^{11} cfu/ml. Phage were allowed to bind for 2-3 h at RT. The wells were then quickly washed 13 times with 300 μ l/well of PBS containing 0.5% Tween-20 (PBST). Bound phage were eluted by incubation with 150 μ l/well of 50 mM glycine-HCl, pH 2.0 for 15 min. The resulting solution was pooled and then neutralized with Tris-HCl, pH 8.0. An equal volume of log-phase TG1 cells were infected with the eluted phage, then plated onto two 24 cm x 24 cm plates containing 2xYT-AG. The plates were incubated at 30°C overnight. The next morning, cells were removed by scraping and stored in 10% glycerol at -80°C. For subsequent rounds of affinity enrichment, cells from these frozen stocks were grown and phage were prepared as described above. A total of 216 clones from the 20mer library and 120 clones from the 40mer library were picked at random from the third and fourth rounds of panning and screened for IR binding activity. DNA sequencing of the clones revealed the abundance of sequences as summarized in Figures 1A, 1B, 2A, 2C, 10A and 10B.

B. One-Day Panning Procedure

Log phase TG1 cells were infected with the eluted phage, amplified in the 2xYT medium for 1 h at 37°C prior to the addition of helper phage, ampicillin and glucose (2% final concentration). After incubation for 1 h at 37°C, the cells were spun down and resuspended in the 2xYT-AK medium. The cells were then returned to the shaker and incubated overnight at 37°C. The overnight phage was then precipitated and subjected to the next round of panning. A total of 96 clones were picked at random from rounds 3 and 4 and screened for binding activity.

To isolate specific binders, each library was panned against a soluble form of the human IR. This IR is composed of the extracellular domains of both the α and β chains of the natural receptor, as well as the constant domain from immunoglobulin Fc, retaining the β - α - α - β structure described above. Because the IR is expressed in a eukaryotic system, disulfide bond

formation and glycosylation patterns should mimic the wild-type receptor. The details of this recombinant protein construct are described in Bass *et al.* (1996).

In panning with the peptide library, the IR was immobilized directly
5 onto a protein-binding plastic surface, and four rounds of panning and
enrichment were carried out. Analysis of phage clones from rounds three
and four showed that 114 of the 216 clones from the 20mer random peptide
library and 17 of the 120 from the 40mer random peptide library bound to IR
(Figures 1A, 1B, 2A, 2C, 4A, 6A, 10A and 10B). Of those clones tested
10 competitively against insulin for receptor binding, all were blocked by the
presence of natural ligand. This result indicated that these phage clones
and insulin bind to the same site (or at least overlapping sites) on IR.

Sequence analysis of several clones shows that there are several
distinct populations, designated as Groups 1 through 8 (Figures 1-8)
15 (Figures 47 and 48). Several of the peptides based on the sequences for
these groups have been chemically synthesized for subsequent testing.
Group 1 (Formula 1 motif) peptides contain the consensus sequence
FYxWF, and are believed to be agonistic in cell-based assays. Group 2
(Formula 6 motif) is composed of two peptides having a consensus
20 sequence VYGR and two cysteine residues each. Thus, Group 2 peptides
are capable of forming a cyclic peptide bridged with a disulfide bond. Group
3 (Formula 2 motif) peptides comprise the preferred consensus sequence F-
Y-x-A/G-L/I-x-x-L (A/G denotes the alanine or glycine residue, and L/I
denotes the leucine or isoleucine residue). Certain Group 3 peptides exhibit
25 agonistic activity in cell-based assays (Figure 49). Neither of these
consensus sequences have any significant linear sequence similarities
greater than 2 or 3 amino acids with mature insulin.

Group 7 (Formula 4 motif) is composed of two exemplary peptides
which do not have any significant sequence homology, but have two
30 cysteine residues 13-14 residues apart, thus being capable of forming a
cyclic peptide with a long loop anchored by a disulfide bridge.

Example 10: ELISA Analyses of Phage

This series of experiments was designed to help characterize the different groups of consensus sequences found during the biopanning of IR. Phage were prepared from each group (two unique sequences each were attempted). Each phage was bound to insulin receptor and competition experiments were performed.

Phage Production. Each phage culture was started by the addition of 30 μ l of the master stock to 20 ml 2xYT-AG in 50 ml centrifugation tubes. Cultures were incubated at 37°C until OD₆₀₀ ~0.6-1.0. M13K07 helper phage were added to a concentration of $\sim 5 \times 10^{10}$ cfu ml⁻¹ and incubated at RT for 30 min. The cultures were centrifuged at $\sim 2500g$ and 4°C for 20 min. The bacterial pellet was resuspended in 30 ml 2xYT-AK. The culture was transferred into 250 ml bottles and incubated O/N at 37°C. The culture was centrifuged at $\sim 2500g$ and 4°C for 20 min (in 50 ml centrifuge tubes). The supernatant was transferred to new 50 ml centrifuge tubes.

Phage ELISA. Each well of the Nunc-Immuno™ plates with the MaxiSorp™ surface were coated with either 50 μ l of 2 ng/ μ l either IR or sIGF-1R in PBS overnight at 4°C. The wells were blocked with 200 μ l of MPBS for 1.5 h at RT. Phage were added at 100 μ l per well. Peptides were added as noted below and allowed to incubate at RT for 3 h. The plates were washed 3 times with PBST. A solution of 1:3000 diluted HRP:Anti-M13 conjugate at 100 μ l per well of was added for 1 h. Following a repeat of the washing, 100 μ l of ABTS was added for 15-30 min. The OD was measured using a SpectraMax 340 Microplate Spectrophotometer (Molecular Devices) at 405 nm.

Peptide Competition. Competition of phage displayed peptides by the addition of soluble peptides was carried out using the phage ELISA as described above. Twenty microliters of the stock synthetic-peptide solution was added to Row A. A series of 20 μ l into 100 μ l dilutions were performed until Row G. Twenty microliters were discarded from Row G to maintain 100 μ l per well. Row H was reserved as no peptide wells. The starting

concentration of the B6 peptide was 68 μM for both receptors. For IR, the starting concentration for the C1 peptide was 48.5 μM . Only 2 μl of the C1 peptide were added to Row A of wells containing IGF-1R. Therefore, the starting concentration was 4.9 μM . The volume was maintained by the addition of 18 μl of the phage solution to Row A.

Natural Ligand Competition. The "Phage First" experiments were performed by adding 10 μl of 5.5 μM , 550 nM, or 55 nM insulin or IGF-1 in PBS to phage-containing wells in the phage ELISA. The working concentrations were 500 nM, 50 nM, and 5 nM. The volume of no ligand wells was maintained by the addition of 10 μl PBS.

The "Ligand First" experiments were performed by added 50 μl of 2 μM , 200 nM, or 20 nM insulin or IGF-1 in PBS containing 0.5% Tween-20 to non-phage containing wells and allowed to incubate 15 min. Fifty microliters of the phage solution was then added to the wells and mixed well. The mixture was allowed to incubate for 2 h at RT and continue with the phage ELISA.

The data are provided in Table 7 and Figures 50A-50D. Sequences were confirmed on all clones by DNA sequencing.

TABLE 7: Phage Characterization Summary

	Absorbance Values			IR Competitions		siGF-1R Competitions	
	NFM	siGF-1R	IR	C1	B6	C1	B6
Group 1							
20D3	0.09	2.26	1.29	Y	Y	-	
B8	0.10	2.55	1.30	Y	Y	-	
Group 2							
20A4	0.15	0.21	1.61	N	N	-	-
D8	0.09	2.19	1.42	N	N	Y	Y
Group 3							
20E2	0.11	2.15	1.01	Y	Y	-	
Group 4							
D10	0.12	0.14	0.73	N*	N	-	-
A2	1.35	2.00	1.79	N	N	N	
Group 5							
D9-2	1.02	2.53	1.64	N	N	-	-
H4	1.16	1.14	1.41	N*	N	-	-
Group 6							
E8	0.10	2.00	1.34	Y	Y	-	
F2	0.09	2.08	1.43	Y	Y	-	
Group 7							
F8	0.14	2.06	1.49	N	N	Y	Y
Group 8							
40A2	0.56	0.55	1.90	Y*	Y	-	-
40H4	0.75	0.83	0.84	-	-	-	-

NFM = Non-fat milk

- 5 C1 peptide (D112) has the FYX₃WF Formula 1 motif and an amino acid sequence of DYKDCWARPCGDAAIFYDWFVQQASKK

B6 peptide has the FYX₈X₉LX₁₁X₁₂L Formula 2 motif and an amino acid sequence of WNTVDPFYHKLSELLREKK

Observations and Conclusions

1. The C1 and B6 peptides bind to IR. The C1 and B6 peptides expressed as phage-displayed peptides are negatively charged.
2. Groups 1, 3, and 6 (Formulas 1, 2 and 10, respectively), appear to be inhibited by both the C1 and B6 peptides when binding to IR and IGF-1R. All three groups behave with similar characteristics and similar affinities. They all bind to a common site, (Site 1) as shown by competition data.
3. Group 2 (Formula 6 motif) phage clones have different properties despite their sequence similarity. The phage 20A4 is an IR-specific clone. Its binding to IR is not inhibited by C1 or B6 peptides and therefore binds to Site 2. The phage D8 binds to both IR and IGF-1R. Inhibition by C1 peptide and B6 peptide occurs only when binding to IGF-1R. D8 is more sensitive to C1 and B6 peptide inhibition than Group 1, 3, and 6, suggesting an allosteric competition.
4. Some phage appear to have a plastic-binding component (binding to the wells of microtiter plates) in their sequences when high amounts of phage are used. The phage A2, D9-2, H4, 40F10, 40A2, and 40H4 have a significant background to their signals. With the exception of 40H4, all signals increase over this background signal in the presence of IR. The signals for phage A2 and D9-2 also increase over background for IGF-1R. It should be noted the phage for the IGF-1R binder B6 shows this similar characteristic.
5. The Group 2 phage 20A4 and Group 4 phage D10 are specific for IR – there is no detectable binding to IGF-1R. D10 may be inhibited by C1 peptide to a small extent.
6. The phage for Group 7, F8 (Formula 4 motif) has characteristics similar to Group 2, D8 (Formula 6 motif). This clone binds to both IR and IGF-1R, but the C1 and B6 peptides only affect D8 binding when bound to IGF-1R. F8 is more sensitive to C1 and B6 peptide inhibition than Group 1, 3, and 6, (Formula 1, 2 and 10 motifs, respectively).

Example 11: Cross-Reactivity Studies

- Phage ELISA experiments show that the IGF-1R peptides H2 and E4 have detectable binding to IR while expressed as a phage fusion. Other IGF-1R-specific peptides such as A6, C1, B6, and JBA5 do not have detectable binding to IR when expressed as phage.

A. Experimental Procedures

- Phage Production.* Each phage culture was started by the addition of 40 μ l of the MASTER stock to 20 ml 2xYT-AG in 50 ml centrifugation tubes. Cultures were incubated at 37°C until OD₆₀₀ ~0.6-1.0. M13K07 helper phage were added to a concentration of $\sim 5 \times 10^{10}$ cfu/ml and incubated at RT for 30 min. The cultures were centrifuged at $\sim 2500g$ and 4°C for 20 min. The bacterial pellet was resuspended in 20 ml 2xYT-AK and incubated O/N at 37°C. The culture was centrifuged at $\sim 2500 \times g$ and 4°C for 20 min. The supernatant was transferred to new 50 ml centrifuge tubes
- Phage ELISA.* Each well of the Nunc-Immuno™ plates with the MaxiSorp™ surface were coated with 50 μ l of 2 ng/ μ l either IR or IGF-1R in PBS O/N at 4°C. The wells were blocked with 200 μ l of 2% (w/v) Carnation non-fat dry milk in PBS for 1.5 h at RT. Phage were added at 100 μ l per well. Peptides were added as noted below and allowed to incubate at RT for 3 h. The plates were washed 3X with PBST. A solution of 1:3000 diluted HRP:Anti-M13 Conjugate at 100 μ l per well of was added for 1 h. Following a repeat of the washing, 100 μ l of ABTS was added for 15-30 min. The OD₄₀₅ was measured using a SpectraMax 340 Microplate Spectrophotometer.

- Peptide Competition.* Peptide Competition Curves were produced during the phage ELISA across rows in triplicate. The stock synthetic peptide solution was added to Column 12 so that the total volume totaled 150 μ l (additional phage solution was added when necessary). A serial dilution was made by transferring 50 μ l from Column 12 into 100 μ l in Column 11, 50 μ l from Column 11 into 100 μ l in Column 10, and continuing

the serial dilution until Column 2. Fifty microliters were discarded from Column 2 to maintain 100 μ l per well. Column 1 was reserved as no peptide wells. The starting working concentrations for each peptide was: H2 – 50 μ M; H2C – 100 μ M; C1C – 100 μ M; D2C – 100 μ M; E4 – 33.3 μ M; C1 – 50 μ M; A6 – 100 μ M; and p53 – 100 μ M.

B. IGF-1R Peptide Competition

An experiment was designed to ascertain whether IGF-1R peptides have the ability to compete phage that bind to IR. Competition will occur in either IR- or IGF-1R-coated wells. The IGF-1R peptides H2, H2C, C1C, D2C, E4, C1, and A6 were tested for competition with two separate phage. The first, 20D3, (Figures 51A, 51C) is a phage discovered during panning of IR, but is also positive for binding to IGF-1R. The second, H2, (Figures 51B, 51D) is a phage found during panning of the IGF-1R, but is also positive for binding to IR. A p53-like peptide that binds to MDM2 was used as a negative control.

The Hill Plot data are provided in Table 8 below, and presented graphically in Figures 52A-52D.

TABLE 8: Hill Plot Data

Pept.	IGF Receptor						Insulin Receptor					
	20D3 Phage			H2 Phage			20D3 Phage			H2 Phage		
	n	K _d	r ²	n	K _d	r ²	n	K _d	r ²	n	K _d	r ²
H2	1.29	4958	0.991	1.21	9812	0.979	1.07	1133	0.978	0.71	762	0.981
H2C	0.81	5055	0.975	1.02	3720	0.987	1.03	564	0.976	0.62	480	0.926
C1	1.37	19	0.988	0.96	40	0.976	0.83	324	0.999	0.46	132	0.922
C1C	1.32	13475	0.990	1.00	34198	0.945	0.70	1190	0.988	0.53	532	0.956
D2C	1.50	12454	0.995	1.34	33124	0.999	0.81	2491	0.995	0.96	2964	0.983
E4	1.53	6522	0.995	1.11	5868	0.961	0.79	1435	0.979	0.71	387	0.994

C. Observations and Conclusions

a. These peptides can bind to IR and inhibit binding of phage found by either panning IR (20D3) or IGF-1R (H2). This crossover event

between the two receptors occurs despite negative results of many of these same phage-displayed peptides.

- b. Although the C1 peptide is the most potent inhibitor of phage binding, C1 peptide loses much of its potency advantage over the other peptides binding IR instead of IGF-1R. In addition, A6 gains potency when binding to IR relative to the other peptides. Combined, this suggests that the adjacent surfaces to this active site of the receptors are sufficiently different that peptides and small organic molecules specific for either receptor can be found.
- c. The Hill Coefficient of the peptides binding to IGF-1R is always 1.5 to 2-fold higher than the same phage and peptide binding to IR.

Example 12: Competition of Phage Binding with Insulin

- Many different peptides isolated from the random peptide libraries were tested for the ability to compete the natural ligand insulin. Clones tested were B8 (D103) (Formula motif 1), F4 (Formula motif 1), A7 (D122) (20A4) (Formula motif 6), D8 (D123; data not shown) (Formula motif 6), C6 (Formula motif 2), E8 (Formula motif 10), H4 (group 5; data not shown), A4 (group 6), G8 (group 7), G7 (Fc binder). H4 most likely binds non-specifically to the material from which the microtiter plate is made.

A. Insulin Competition Procedure

- Receptors were coated at 100 µg/ml, 50 µl/well. After blocking with MPBS and washing 3x with PBST, insulin was added in the presence of 0.1% Tween-20 at 2 µM, 100 nM, and 5 nM for 15 min before the addition of IR binding phage. The final concentration of insulin was 1 µM, 50 nM and 2.5 nM. Reaction was incubated at RT for 1 h and wells were washed 3x with PBST (PBS with 0.05% Tween-20). Anti-M13 HRP was added and incubated for 1 h at RT. Wells were washed 3x with PBST before the addition of ABTS. Plates were read at 405 nm.

B. Results

At high insulin dosage, all clones, except F4, G7, and H4 # (not shown), were inhibited; B8 showed the best inhibition, >50%. The apparent lack of binding of F4 (group 1) might be due to the insufficient level of phage present. G7 is a Fc binding phage is should not by inhibited by insulin. H4 is suspected to be a plastic-binding phage. The results are presented in Figure 54.

C. Conclusions

Insulin competition with a representative member from each group indicated that almost all of the groups competed with insulin; only the "plastic binders" and Fc binding phage did not compete. Different degrees of inhibition by these peptides (phage) imply that the peptides recognize different epitopes on or in the close proximity of the receptor active site.

Example 13: Synthetic Peptide 20A4 Competition Results

This experiment was performed as in Example 12. The 20A4 peptide (D122) starting concentration was 58 μ M.

Results. The results are included in Table 7. The peptide 20A4 (D122) (A7) competes with Group 2 members (Formula 6 motif), Group 4 member (miscellaneous) D10, and Group 7 member (Formula 4 motif) F8 (D124). There is a partial inhibition of Group 6 member F2. The data is consistent with the conclusion that the site for 20A4 binding is different from the site for Group 1, Group 3, and Group 6.

Example 14: Peptide Fusions to the Maltose Binding Protein - Construction, Purification and Characterization of the Binding to the Insulin Receptor

A. Cloning

5 The transfer of interesting peptide sequences from phage display to display as maltose binding protein (MBP) -fusions is desirable for several reasons. First, to obtain a more sensitive affinity estimate, the polyvalency of phage display peptides should be converted to a monovalent system. For this purpose, the peptide sequences are fused to MBP that generally exists
10 as a monomer with no cysteine residues. Second, competition experiments can be carried out with the same or different peptides, one phage displayed and the other fused to MBP. Lastly, purified peptides can be obtained by cleavage of the fusion protein at a site engineered in the DNA sequence.

Figure 55 shows a schematic drawing of the MBP-peptide construct.
15 In the construct, the N-terminus of the peptide sequence is fused to the C-terminus of the MBP. Two peptide-flanking epitope tags are included, a shortened-FLAG at the N-terminus and E-Tag at the C-terminus. The corresponding gene fusion was generated by ligating a vector fragment encoding the MBP in frame with a PCR product encoding the peptide of
20 interest. The vector fragment was obtained by digesting the plasmid pMAL-c2 (New England Biolabs) with *EcoRI* and *HindIII* and then treating the fragment with shrimp alkaline phosphatase (SAP; Amersham). The digested DNA fragment was resolved on a 1% agarose gel, excised, and purified by QIAEXII (QIAGEN). The 20-amino acid peptide sequences of
25 interest were initially encoded in the phage display vector pCANTAB5E (Pharmacia). To obtain these sequences, primers were synthesized which anneal to sequences encoding the shortened FLAG or E-Tag epitopes and also contain the required restriction enzyme sites *EcoRI* and *HindIII*. PCR products were obtained from individual phage clones and digested with
30 restriction enzymes to yield the insert fragment. The vector and insert were ligated overnight at 15°C. The ligation product was purified using QIAquick

- spin columns (QIAGEN) and electroporations were performed at 1500 v in an electroporation cuvette (0.1 mm gap; 0.5 ml volume) containing 10 ng of DNA and 40 μ l of *E. coli* strain ER2508 (RR1 *lon::miniTn10*(Tet^r) (*malB*) (*argF-lac*)*U169* Pro⁺ *zjc::Tn5*(Kan^r) *thiA2*) electrocompetent cells (New England Biolabs). Immediately after the pulse, 1 ml of pre-warmed (40°C) 2xYT medium containing 2 % glucose (2xYT-G) was added and the transformants were grown at 37°C for 1 h. Cell transformants were plated onto 2xYT-AG plates and grown overnight at 37°C. Sequencing confirmed the clones contained the correct constructs.

10 **B. Small-Scale Expression of Soluble MBP-Peptide Fusion Proteins**

- E. coli* ER2508 (New England Biolabs) carrying the plasmids encoding MBP-peptide fusion proteins were grown in 2xYT-AG at 37°C overnight (250 rpm). The following day the cultures were used to inoculate media (2x YT containing-G) to achieve an OD₆₀₀ of 0.1. When the cultures reached an OD₆₀₀ of 0.6, expression was induced by the addition of IPTG to a final concentration of 0.3 mM and then cells were grown for 3 h. The cells were pelleted by centrifugation and samples from total cells were analyzed by SDS-PAGE electrophoresis. The production of the correct molecular weight fusion proteins was confirmed by Western blot analysis using the monoclonal antibody anti-E-Tag-HRP conjugate (Pharmacia).

C. Large-Scale Expression of Soluble MBP-Peptide Fusion Proteins

- E. coli* ER2508 carrying plasmids encoding the MBP-peptide fusion proteins were grown in 2xYT-AG media for 8 h (250 rpm, 37°C). The cultures were subcultured in 2xYT-AG to achieve an OD₆₀₀ of 0.1 and grown at 30°C overnight. This culture was used to inoculate a fermentor with medium of following composition (g/l):

	Glucose	3.00
	(NH ₄) ₂ SO ₄	5.00
	MgSO ₄ · 7H ₂ O	0.25
	KH ₂ PO ₄	3.00
5	Citric Acid	3.00
	Peptone	10.00
	Yeast extract	5.00
	pH 6.8	

- 10 The culture was grown at 700 rpm, 37°C until the glucose from the medium was consumed ($OD_{600} = \sim 6.0 - 7.0$). Expression of the fusion protein was induced by the addition of 0.3 mM IPTG and the culture was grown for 2 h in fed-batch mode fermentation with feeding by 50 % glucose at a constant rate of 2 g/l/h. The cells were removed from the medium by
- 15 centrifugation. Samples of the cell pellet were analyzed by SDS-PAGE followed by the Western blot analysis using the mouse monoclonal antibody anti-E-Tag-HRP conjugate (Pharmacia) to visualize the expressed product.

D. Purification

- The cell pellets were disrupted mechanically by sonication or
- 20 chemically by treatment with the mild detergent Triton X-100. After removal of cell debris by centrifugation, the soluble proteins were prepared for chromatographic purification by dilution or dialysis into the appropriate starting buffer. The MBP fusions were initially purified either by amylose affinity chromatography or by anion exchange chromatography. Final
- 25 purification was performed using anti-E-Tag antibody affinity columns (Pharmacia). The affinity resin was equilibrated in TBS (0.025 M Tris-buffered saline, pH 7.4) and the bound protein was eluted with Elution buffer (100 mM glycine, pH 3.0). The purified proteins were analyzed for purity and integrity by SDS-PAGE and Western blot analysis according to standard
- 30 protocols.

For BIAcore analysis of fusion protein and synthetic peptide binding to insulin receptor, insulin (50 µg/ml in 10 mM sodium acetate buffer pH 5) was immobilized on the CM5 sensor chip (Flowcell-2) by amine coupling.

Flowcell-1 was used for background binding to correct for any non-specific binding. Insulin receptor (450 nM) was injected into the flow cell and the binding of IR to insulin was measured in resonance units (RUs). Receptor bound to insulin gave a reading of 220 RU. The surface was regenerated with 25 mM NaOH. Pre-incubation of receptor with insulin in a tube at RT completely abrogated the response units to 16 RU. Thus, the system was validated for competition studies. Several maltose-binding fusion proteins, peptides and rVabs were pre-incubated with insulin receptor before injecting over the insulin chip for competition studies. The decrease in binding/resonance units indicates that several MBP-fusion proteins can block the insulin binding site. The results are shown in Tables 9 and 10. The amino acid sequences referred to in the tables are identified in Figures 47 and 48, except the 447 and 2A9 sequences, which are shown below.

TABLE 9: BIAcore Results—Fusion Proteins Compete for Binding to IR

	Incubation Mixtures	Result (RUs)	Sequence Type
Controls	Insulin Receptor (IR) 450 nM	220	Positive Control
	Insulin (8.7 μ M)	16	Negative Control
MBP Fus. Prots.	A7 (20A4)-MBP (4.1 μ M) + IR	43	Formula 6 Motif
	D8-MBP (1.6 μ M) + IR	56	Formula 6 Motif
	D10-MBP (3.4 μ M) + IR	81	Formula 11 Motif
	447-MBP (11.5 μ M) + IR	195	hGH Pept. Fus.
	MBP (13 μ M) + IR	209	Negative Control

TABLE 10: BIAcore Results—Synthetic peptides compete for binding to IR

Incubation Mix	% Binding	Result (RUs)	Sequence Type
IR	100	128	Positive control
IR + 20D1	41	51.8	Formula 1 Motif
IR + D8	33	41.6	Formula 6 Motif
IR + 20C11	38	49	Formula 2 Motif (bkg high)
IR + H2	27	34.6	IGF (phosphorylated band)
IR + 2A9	100	128	IGF(bkg high)
IR + 20A4	33	41.8	Formula 6 Motif
IR + p53wt	97	124.5	P53 wild type

The concentration of each peptide was about 40 μ M and the concentration of IR was 450 nM. The 447 peptide sequence is: LCQRLGVGWPGWLSGWCA. The 2A9 peptide sequence is: LCQSWGVRIGWLTGLCP.

Example 15: Insulin Receptor Competition ELISA Using Phage Displayed Peptides and MBP-Peptide Fusion Proteins

To determine whether the binding sites (contact sites) on the insulin receptor for the various peptides are similar, the purified fusion proteins were used in ELISA competition experiments with phage displayed peptides from various groups. Phage-displayed peptides, which were able to bind to IR, were classified into various groups according to consensus sequences identified (see Figures 47 and 48). Peptide sequences of interest were fused to the C-terminus of MBP as previously described. The protein fusion constructs were expressed as soluble proteins, purified, and the protein concentrations were determined. The purified fusion proteins were used in ELISA competition experiments with phage displayed peptides from the various groups as shown in Table 11.

As expected, the fusion proteins containing A7 (20A4), D8, D10, and F8 peptides were able to compete the corresponding identical peptide sequence displayed on phage in the range of 28-54% of the control value. The fusion protein, MBP-A7, was able to significantly compete (<54%) phage-displayed peptides D8, D10, and F8. The other fusion protein from Group 2 (Formula 6 motif), MBP-D8, was able to compete A7 and D10 peptides displayed on phage. Furthermore, the Group 7 (Formula 4 motif) fusion protein MBP-F8 competed A7 and D10 phage displayed peptides. Figures 56A and 56B show the plotted data from Table 11. In Figure 56A, a clear pattern is seen where significant ($\leq 54\%$) competition reactions occur between fusion proteins and phage-displayed peptides which have in common the presence of at least two cysteine residues (see Figures 47 and 48 for peptide sequences).

Also striking is the observation that the cysteine containing fusion proteins were not able to compete phage displayed peptides from Group 1 (Formula 1 motif), which contain the consensus (IGF A6-like) sequences and are without cysteine residues (Figure 56A). In Figure 56B, the fusion proteins containing the Group 1 (Formula 1 motif) consensus sequences

were not able to compete to a significant extent any of the phage-displayed peptides from any of the groups. It should be noted that the corresponding identical phage from Group 1 was not tested. The data support the conclusion that the cysteine-containing peptides bind to a contact site (Site 2) which is different than the contact site (Site 1) required for the consensus containing peptides (Group 1, (Formula 1 motif)) to bind the insulin receptor.

TABLE 11

Phage Displayed		Group 1		Group 2		Group 4	Group 7	Control
Peptides		MBP-E7 1.6 μ M	MBP-H8 1.6 μ M	MBP-A7 (20A4) 5 μ M	MBP-D8 2 μ M	MBP-D10 4 μ M	MBP-F8 2.8 μ M	MBP-447 14 μ M
Group 1	B8	265	264	329	267	274	240	299
	20 D3	196	196	250	170	218	208	186
Group 2	D8	138	135	<u>53</u>	<u>54</u>	129	111	160
	A7 (20A4)	133	103	<u>28</u>	<u>54</u>	125	<u>21</u>	116
Group 3	20 E2	80	106	100	69	84	161	100
Group 4	A2	92	92	88	74	105	98	79
	D10	92	60	<u>20</u>	<u>20</u>	<u>36</u>	<u>20</u>	117
Group 6	F2	91	97	88	83	92	83	101
	E8	86	75	164	99	94	86	110
Group 7	F8	99	93	<u>44</u>	63	82	<u>43</u>	138
Group 8	40 A2	80	74	118	84	95	80	90

Data reported in the table above was obtained as follows: IR was coated on a 96-well plate with 50 μ l of 2 ng/ μ l IR and incubated overnight at 4°C. The wells were then blocked with MPBS for 1 h. The fusion proteins (mixed #1:5 with MPBS) were added to the wells and incubated at RT for 30 min. An equal volume of phage (displaying various peptides from each of the groups) was then added and incubated for 1.5 h. The control well contained only phage and an equal volume of buffer. The plate was washed 3 times in PBST and then incubated with HRP/anti-M13 conjugate for 45 min. The plate was washed again and then the ABTS substrate added. The

values indicate readings taken at OD₄₀₅ which were normalized as percent control. The control fusion protein MBP-447 contains a peptide that binds the growth hormone receptor. Peptides in bold type contain cysteine residues. Underlined and in bold are values which are $\leq 54\%$ of control values.

Example 16: Biopanning the rVab Library

The same rVab library described in Example 5 and panned for members that bound IGF-1R was also panned for members that bind IR. Human insulin receptor was diluted to 1 mg/ml in 50 mM sodium carbonate buffer, pH 9.5. One hundred microliters of this solution was added to an appropriate number of wells in a 96-well microtiter plate (MaxiSorp plates, Nunc) and incubated overnight at 4°C. The wells were then blocked by adding 100 μ l of MPBS to each well and incubating at RT for 1 h.

The phage were incubated with MPBS for 30 min at RT, then 100 μ l of the phage solution were added to each well and incubated for 2 h at RT. In the first round, the input phage titer was about 10^{13} cfu/ml. The input phage titer was about 10^{11} cfu/ml in subsequent rounds.

The wells were washed 13 times with 200 μ l/well of MPBS, then washed once with PBS (200 μ l/well). The bound phage were eluted by adding to each well 100 μ l of 20 mM glycine-HCl, pH 2.2. After 30 s, the phage was transferred to an Eppendorf tube and the solution was neutralized by adding 50 μ l of 1 M Tris-HCl, pH 8.0, per volume from each well.

TG1 cells were grown to the mid-log phase (OD₆₀₀ = 0.5). Equal volumes of the TG1 cell culture and the neutralized phage solution were mixed together, incubated for 1 h at 37°C without shaking, and then plated onto two 24 cm x 24 cm 2xYT-AG agar plates. The next morning, cells were removed by scraping the surface of the agar plates, and were then suspended in 24 ml 2xYT and stored in 10% glycerol at -80°C.

The input phage for the subsequent rounds of biopanning was prepared by growing 100 μ l of the cells from these frozen stocks, followed

by phage preparation according to the Protocol Preparation of Phage described below.

Protocol: Preparation of Phage

- 5 The general protocol for phage preparations used to prepare phage displayed rVabs is described below.
1. Phagemid-containing TG1 cells were grown to $OD_{600} = 0.5$ in 2xYT-AG media at 37°C with shaking (250 rpm).
 2. M13K07 helper phage were then added (at MOI = 20), and the cells were incubated for 1 h at 37°C with gentle shaking (150 rpm).
 - 10 3. Following infection, cells were pelleted by centrifugation at 1,000 g for 20 min and the supernatant containing the helper phage were discarded.
 4. The cell pellet was resuspended in the initial culture volume in 2xYT-AK and grown overnight at 30°C with shaking (250 rpm).
 - 15 5. The cells from the overnight culture were pelleted at 3,000 g for 30 min at 4°C and the supernatant containing the phage was recovered.
 6. The supernatant was adjusted to contain 4% PEG, 500 mM NaCl and chilled on ice for 1 h. The precipitated phage was pelleted by centrifugation at 10,000 x g for 30 min. The pellet was resuspended in
 - 20 MPBS.

Example 17: Expression and Characterization of IR Binding rVab Clones

A. Infection of *E. coli* HB2151 Cells

- a. To prepare the log-phase cells, 2xYT media was inoculated
- 25 with *E. coli* strain HB2151 cells (genotype) from a fresh minimal medium plate, and the cells were grown to $OD_{600} = 0.5$ at 37°C with shaking (250 rpm).

b. Fifty microliters of the pool phage from biopanning round 3 (or round 4) were transferred to 2 ml of the log phase HB2151 cells. The cells were incubated with gentle shaking for 1 h at 37°C.

- c. The cells were diluted appropriately with the 2xYT media,
5 plated on 2xYT-AGN plates and incubated overnight at 30°C.

B. Preparation of Soluble Antibodies for Screening IGF Repetition

- a. Four hundred microliters of 2xYT-AG media were added to each cluster tube (in a rack of 96 tubes in a microtiter format, Costar
10 #4411).

- b. The media in cluster tubes were inoculated by transferring the individual well-isolated colonies from the 2xYT-AGN plates using sterile toothpicks; the cluster tubes were then incubated overnight at 30°C with shaking (250 rpm). The array of bacterial cultures in cluster tubes
15 constitutes the Master Plate.

- c. The next day, the Master Plate was duplicated by transferring 40 µl of the saturated culture from each tube of the Master Plate to 400 µl of 2xYT-AG medium in a new set of cluster tubes. The new array of duplicated cultures in the microtiter plate format was labeled S1.

- d. Plate S1 was incubated for 2 h at 30°C with shaking (250 rpm),
20 and then centrifuged at 1,000 X g for 20 min at RT in a centrifuge equipped with microtiter plate adapters.

- e. The supernatant was carefully removed from each cluster tube and discarded to an appropriate waste container. Four hundred microliters of the 2xYT-AI medium (no glucose added) was added to each tube in plate
25 S1, and the plate was incubated overnight at 30°C with shaking (250 rpm).

- f. Plate S1 was centrifuged as described above, and 320 µl of each supernatant (containing the soluble recombinant antibodies) was carefully transferred to a corresponding tube in a new set of 96 cluster
30 tubes. The new plate was labeled S2.

g. Eighty microliters of the MPBS blocking buffer was added to each tube of plate S2 (already containing 320 μ l of the supernatant) and incubated for 10 min at RT. This rVab preparation was now ready to be used in an ELISA performed described above.

5 **C. Detection of rVab Binding Using
 HRP/Anti-E-Tag Conjugate**

a. A microtiter plate was coated with the target protein and blocked as previously described. Some of the wells of the microtiter plate were coated with an unrelated antigen to serve as a negative control.

10 b. The rVab preparation prepared above was diluted two-fold with the MPBS blocking buffer. Two hundred microliters of this solution was added to a set of antigen-coated and control wells.

c. The plate was incubated for 2 h at RT, and then washed 3 times with PBST.

15 d. The HRP/Anti-E-Tag conjugate was diluted 1:4,000 in the MPBS blocking buffer. Two hundred microliters of the diluted conjugate was added to each well, and the plate was incubated for 1 h at RT.

e. The microtiter plate was washed 3 times with PBST.

20 f. Two hundred microliters of the ABTS solution was added to each well, the microtiter plate was incubated for 20 min at RT, and the absorbance of each well was read at 405 nm in an appropriate microtiter plate reader.

D. Production of Soluble rVabs

25 a. A suitable rVab clone in HB2151 cells was transferred from a 2xYT plate to 3 ml of 2xYT-AG media, and the culture was incubated overnight at 30°C with shaking (250 rpm).

b. Part of the overnight culture (2.5 ml) was added to 25 ml of the 2xYT media and incubated for 1 h at 30°C with shaking (250 rpm).

30 c. The culture was centrifuged at 1000 g for 20 min at RT, and the supernatant was removed from the pelleted cells and discarded.

pelleted cells were resuspended in 25 ml of 2xYT-AI media (no glucose is added) and were incubated overnight at 30°C with shaking (250 rpm).

E. Purification of rVabs

5 The Pharmacia RPSA Purification Module kit was used (Cat. #17-1362-01), and purification was performed according to the manufacturer's directions.

a. A syringe was filled with the Elution Buffer (100 mM glycine, pH 3.0).

10 b. The stopper on the top of the anti-E-Tag column was removed and a drop of the Elution Buffer was added to the top of the column. The syringe was connected to the column with the Luer adapter. The connection was "drop to drop" to avoid introducing air into the column.

15 c. The twist-off end was removed and the column was washed with 15 ml of the Elution Buffer at a flow rate of 5 ml/min, followed immediately by 25 ml Binding Buffer (10X Binding Buffer: 0.20 M Phosphate Buffer, 0.05% NaN₃, pH 7.0).

d. Sample was applied with a peristaltic pump P-1 (Pharmacia, Cat. #19-4611-02) at a flow rate of 5 ml/min at 4°C.

20 e. The column was washed with 25 ml of the Binding Buffer at a flow rate of 5 ml/min to remove unbound *E. coli* proteins.

f. Bound rVab was eluted from the anti-E-Tag column with the Elution Buffer. The first 4.5 ml of material eluted from the column was discarded.

25 g. The next 5 ml (containing the purified E-tagged rVab) was collected in either one or several fractions.

h. The column was immediately re-equilibrate with 25 ml of the Binding Buffer for use with the next sample.

Example 18: Competition ELISA with rVabs

30 For IC₅₀ determinations, microtiter plates were coated with IR and blocked as in Example 9. Soluble rVabs were prepared as described in

- Example 9. Prior to addition of soluble rVabs to the plates, 100 I/well of 100 nM insulin solution in PBS was added to duplicate wells. After incubation for 1 h at RT, the prepared soluble rVabs were added to each well (100 µl/well) without removing the insulin solution. After incubation for 1 h at RT, the wells were washed and the color was developed as described in Example 9.

Example 19: Activities of rVabs in the Cell-Based Assay

- Agonistic and antagonistic activities of IR-specific soluble rVabs were tested in 969 cells stably transfected with the gene encoding the human IR and IRS-1 (insulin receptor substrate). The resulting cell line requires IL-3, IL-4, or insulin for growth. Negative control cell lines do not require IRS-1 for growth. The cells were grown in RPMI 1640 media containing 10% FCS and 20 units of IL-3 per ml. Cells were seeded at 30,000 cells/well in 50 µl PRMI1640 (without IL-3) media containing horse serum instead of FCS to reduce the background. Fifty microliters of either insulin or soluble rVabs at different concentrations were added to duplicate wells, followed by incubation for 18 h in a CO₂ incubator. The cell proliferation assays were performed using WST-1 reagent. The WST-1 tetrazolium salt is cleaved to form formazan by the succinate-tetrazolium reductase system that is active only in viable cells. An increase in the number of cells results in an increase of the overall enzymatic activity of the dehydrogenase that results in a higher absorbance at 450 nm. Ten microliters of WST-1 reagent were added and the plate was incubated for 1-4 h at 36°C. Figure 60 shows the results of these studies. As can be seen, rVab 12h10 was able to induce an agonist response in 32D cells expressing IR with an ED₅₀ of approximately 50 nM.

Example 20: IR Activation Assays

The kinase receptor activation ELISA is a functional assay based on the ability of a sample to stimulate or inhibit autophosphorylation of the insulin receptor construct that has been transfected into 32D cells (Wang et

al., 1993; clone 969). The assay procedure begins with the cell stimulation. The IR transfected 32D cells were seeded at 5×10^5 cells/well in 96-well tissue culture plates and incubated overnight at 37°C. Samples were diluted 1:10 in the stimulation medium (PRIM1640 with 25 nM HEPES pH 7.2) plus
5 or minus insulin. The culture media was decanted from the cell culture plates, and the diluted samples were added to the cells. The plates were incubated at 37°C for 30 min. The stimulation medium was decanted from the plates, and cell lysis buffer (50 mM HEPES pH 7.2, 150 mM NaCl, 0.5% Triton X-100, 1 mM AEBSF, 10 KIU/ml aprotinin, 50 µM leupeptin, and 2 mM
10 sodium orthovanadate) was added. The cells were lysed for 30 min.

In the ELISA portion of the assay, the cell lysates were added to the BSA-blocked anti-IR unit mAb (Upstate Biotechnology, Lake Placid, NY) coated ELISA plates. After a 2 h incubation, the plates were washed 6 times with PBST and biotinylated anti-phosphotyrosine antibody (Upstate
15 Biotechnology) is added. After another 2 h incubation, the plates were again washed 6 times. Streptavidin-Eu was then added, and the plates were incubated for 1 h. After washing the plates again, EG&G Wallac enhancement solution (100 mM acetone-potassium hydrogen phthalate, pH 3.2; 15 mM 2-naphthyltrifluoroacetate; 50 mM tri(n-octyl)-phosphine oxide;
20 0.1% Triton X-100) was added into each well, and the plates were placed onto a shaker for 20 min at RT. Fluorescence of samples in each well was measured at 615 nm using a VICTOR 1420 Multilabel Counter (EG&G Wallac).

Alternatively, IR autophosphorylation was determined using a
25 holoenzyme phosphorylation assay. In accordance with this assay, 1 µl of purified insulin receptor (isolated from a Wheat Germ Agglutinin Expression System) was incubated with 25 nM insulin, or 10 or 50 µM peptide in 50 µl autophosphorylation buffer (50 mM HEPES pH. 8.0, 150 mM NaCl, 0.025% Triton-X-100, 5 mM Mn₂Cl, 50 µM sodium orthovanadate) containing 10 µM
30 ATP for 45 min at 22°C. The reaction was stopped by adding 50 µl of gel loading buffer containing β-mercaptoethanol (Bio-Rad Laboratories, Inc.,

- Hercules, CA). The samples were run on 4-12% SDS-polyacrylamide gels. Western Blot analysis was performed by transferring the proteins onto nitrocellulose membrane. The membrane was blocked in PBS containing 3% milk overnight. The membrane was incubated with anti-phosphotyrosine 4G10 HRP labeled antibody (Upstate Biotechnology) for 2 h. Protein bands were visualized using SuperSignal West Dura Extended Duration Substrate Chemiluminescence Detection System (Pierce Chemical Co.).

Example 21: Development of IR Assays Using Soluble rVab Antibodies and Biotinylated Peptides

- 10 a. Heterogeneous Time-Resolved Fluorescence Assay. Sixty microliters of insulin receptor (60 ng/well) was coated onto 96-well low-fluorescence MaxiSorp (Nunc) plates overnight at 4°C. The plates were blocked with TBS containing 2% milk and 0.5% BSA for 1 h at RT followed by three TBS washes. To test binding of peptides to insulin receptor, serial
- 15 dilutions of biotinylated peptides were added to IR coated plates for 2 h to overnight. After TBS wash, europium-labeled streptavidin at 1 µg/ml in assay buffer (100 mM Tris-HCl, pH 7.8; 150 mM NaCl; 0.5% BSA; 0.05% bovine Ig; 0.05% NaN₃; 0.01% Tween-20) was added to the plates and incubated for 1 h. To test binding of rVab antibodies to IR, Eu-labeled rVab
- 20 antibodies in assay buffer were added to the plates and incubated for 2 h to overnight. After incubation with Eu-labeled streptavidin (for peptide test) or europium-labeled rVabs, the plates were washed 5 times with Tris-buffered saline (pH 7.5) containing 0.1% Tween-20 (TTBS) and tapped dry. Sixty microliters of EG&G Wallac enhancement solution (100 mM acetone-
- 25 potassium hydrogen phthalate, pH 3.2; 15 mM 2-naphtyltrifluoroacetate; 50 mM tri(n-octyl)-phosphine oxide; 0.1% Triton X-100) was added into each well, and the plates were placed onto a shaker for 20 min at RT. Fluorescence of samples in each well was measured at 615 nm using a VICTOR 1420 Multilabel Counter (EG &G Wallac).
- 30 b. Homogeneous Time-Resolved Fluorescence Assay. A mixture of 27 nM Cy5-labeled rVab 43G7 and 6-8 nM LANCE-labeled IGF-1R

- (EG&G Wallac) in Tris-buffered saline containing 0.1% BSA is added to 96- or 384-well white low-fluorescence plates (Nunc) for 2 h or overnight. For library screening, 20 μ M of small organic molecules in 2 % DMSO are included in the mixture. Unlabeled rVab 43G7 at 50 nM or IGF at 3 μ M are used as positive controls. Fluorescence of samples in each well is measured at both 615 nm and 665 nm using a VICTOR 1420 Multilabel Counter (EG &G Wallac).

Example 22: Binding of Synthetic Peptides to Insulin Receptor

- A series of synthetic peptides were synthesized and biotinylated (Anaspec, Inc., San Jose, CA). The binding affinities of these peptides to IR and IGF-1R were tested. Most of these peptides bind to IR at micromolar range (Figure 63). Comparison of binding of biotinylated C1 peptide to IGF-1R and IR is shown in Figure 64, which indicates that binding of C1 to IGF-1R is at the nM range while binding to IR is at the micromolar range. A series of unlabeled peptides or soluble rVab were added to test competition binding to IR (Figure 65). H2C peptide at 30 μ M appears to compete for binding to IR with biotinylated peptides from group 1 (Formula 1 motif) (20D1 and 20D3) and the two A6-based peptides (C1 and H2) but not compete with peptides from group 2 (Formula 6 motif) (20A4 and D8), group 3 (Formula 2 motif) (20C11) or the IGF peptide A9. The 33 F7 soluble rVab antibody competes with group 1 and 2 peptides as well as C1 peptide, however, it does not compete with 20C11 or 2A9. Figure 66 shows that H2C competition with biotinylated peptides, 20D3, H2, and C1, binding to IR is dose-dependent. C1C peptide also competes with C1 for IR binding (Figure 67).

Example 23: Competition for Binding to rVab 12H10 by Peptides and MBP-Peptide Fusion Proteins

Several peptides and four MBP-peptide fusion peptides were tested for competition of binding to IR with soluble rVab 12H10. Figure 68 shows

that C1 and H2C at 30 μ M inhibit binding to 40-50% of control and C1C at 30 μ M inhibit to 60%. B6 and growth hormone do not compete with binding of 12H10 to IR. Four MBP-peptide fusion proteins (D10, 20A4, E7 and H8) all inhibit binding of 12H10 to IR to 20-30 % of control (Figure 69).

5 **Example 24: Effects of Small Organic Molecules on IR Phosphorylation**

Organic molecules positive for binding to IGF-1R and negative controls can be tested for their effects on phosphorylation of insulin receptor.

10 **Example 25: Method for Determination of Insulin Receptor Binding of Peptides**

In other insulin binding assays, IR was incubated with 125 I-labeled insulin at various concentrations of test substance and the K_d was calculated. According to this method, human insulin receptor (HIR) or human IGF-1 receptor (HIGF-1R) was purified from transfected cells after
15 solubilization with Triton X-100. The assay buffer contains 100 mM HEPES (pH 7.8), 100 mM NaCl, 10 mM MSG, 0.5% human serum albumin, 0.2% gammaglobulin and 0.025% Triton X-100. The receptor concentration was chosen to give 30-60% binding of 2000 cpm (3 pM) of its 125 I-labeled ligand (TyrA14- 125 I-HI or Tyr31- 125 I-IGF1) and a dilution series of the substance to
20 be tested was added. After equilibration for 2 days at 4°C, each sample (200 μ l) was precipitated by addition of 400 μ l 25% PEG 6000, centrifuged, washed with 1 ml 15% PEG 6000, and counted in a gamma-counter.

The insulin/IGF-1 competition curve was fitted to a one-site binding model and the calculated parameters for receptor concentration, insulin
25 affinity, and non-specific binding were used in calculating the binding constants of the test substances. Representative curves for insulin and IGF-1 are shown in Figures 71A-71N.

The sequences of certain peptides analyzed are shown in Table 12, except for peptides D125 and D126. Synthetic peptides are numbered
30 D1XX. D117K is an analog of D117 with an extra N-terminal lysine added

for facilitate solubility. Peptides produced recombinantly by phage are indicated as D1XXA.

The peptides are all biotinylated in the side chain of the C-terminal lysine (except D117A). The peptides produced recombinantly are C-
5 terminal acids, whereas the synthetic peptides are C-terminal amides.

The results of the binding and phosphorylation assays are shown in Table 13.

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TABLE 12

Name	Sequence	Motif
D101	KIGGQGHQDGNFYDWFVEALAKK	1
D102	KVLQARHGCDVSDFYEWFAKK	1
D103	KWSALLSVMDFGYAWFDDAVKK	1
D104	KGHSWALVRHVDRLFYEWFDLKK	1
D105	KRDKPTDQEEQNWSFYEWFRHKK	1
D106	KVFWNCRSQQLDFYEWFEQAANK	1
D107	KLESHYVVPQAALDRLFYSWFSKK	1
D108	KFYGWFSRQLSLTPRDDWGLPKK	1
D109	KSAPGLVSNKQDGLFYSWFREKK	1
D110	KRGGGTFYEWFEFESALRKHGAGKK	1
D111	KDPERMQSDVGFYEFWFAAVGKK	1
D112	DYKDCWARPCGDAANFYDWFVQQASKK	1
D113	DYKDVTFTSAVFHENFYDWFVRQVSKK	1
D114	SAKNFYDWFVKK	1
D115	ADKNFYDWFMAAKK	1
D116	DYKDLQCSWGVRIWLAGLCPKK	9
D117	FHENFYDWFVRQVSKK	1
D117K	KFHENFYDWFVRQVSKK	1
D118	DYKDFYDAIDQLVRGSARAGGTRDKK	2
D119	KDRAFYNGLRDLVGAVYGAWDKK	2
D120	KVRGFQGGTVWPGYEWLRNAKK	10
D121	KSMFVAGSDRWPGYGVLDWLKK	10
D122	KEIEAEWGRVRCCLVYGRCVGGKK	10
D123	KWLDQEWAWVQCEVYGRGCPSKK	6
D124	KHLCVLEELFWGASLFGYCSGKK	4
D101A	KIGGQGHQDGNFYDWFVEALAKK	1
D102A	KVLQARHGCDVSDFYEWFAKK	1
D112A	DYKDCWARPCGDAANFYDWFVQQASKK	1
D113A	DYKDVTFTSAVFHENFYDWFVRQVSKK	1
D117A	FHENFYDWFVRQVSKK	1
D119A	KDRAFYNGLRDLVGAVYGAWDKK	2
D122A	KEIEAEWGRVRCCLVYGRCVGGKK	10
D123A	KWLDQEWAWVQCEVYGRGCPSKK	6
D124A	KHLCVLEELFWGASLFGYCSGKK	4

TABLE 13

Name	K _d (μM) HIR	K _d (μM) HIGF1R	Ratio	Autophosph. Blot
D101	0.51	13	25	-
D102	1.2	7.4	6.2	-
D103	0.74	15	20	-
D104	20	>20		-
D105	2.8	12	4.3	-
D106	0.97	6.2	6.4	-
D107	1.1	9.7	8.8	+
D108	2.3	19	8.3	-
D109	3.6	12	3.3	-
D110	0.84	1.4	1.7	-
D111	0.62	3.2	5.2	-
D112	0.49	0.05	0.1	-
D113	0.75	5.4	7.2	- (prec)
D114	8.1	>20	>2.5	0
D115	8.1	>20	>2.5	0
D116	4.4	8.1	1.8	0
D117	0.70	6.1	8.6	+
D117K	0.82	9.1	11.1	
D118	0.25	1.3	5.2	+
D119	4.5	13	2.9	+
D120	0.37	2.2	5.9	-
D121	1.1	7.4	6.7	-
D122	1.2	>20	>17	0
D123	0.55	16	29	0
D124	0.04*	8.2	200	-
D101A	0.27	11.0	41	
D102A	0.97	16.0	16	
D112A	0.19	0.02*	0.1	
D113A				
D117A	0.60	5.1	8.5	
D119A	3.0	2.5	0.8	
D122A	1.0	>20	>20	
D123A	1.3	>20	>15	
D124A	0.09*	>20	>200	
D125A	2.6	>20	>8	
D126A	1.4	18	13	

**Example 26: Determination of Insulin Agonist Activity
Based On ^3H -Glucose Uptake into Adipocytes**

- Insulin increases uptake of ^3H glucose into adipocytes and its conversion into lipid. Incorporation of ^3H into the lipid phase was determined
- 5 by partitioning of lipid phase into a scintillant mixture, which excludes water-soluble ^3H products. The effect of compounds on the incorporation of ^3H glucose at a sub-maximal insulin dose was determined, and the results expressed as increase relative to full insulin response. The method was adapted from Moody *et al.* (1974).
- 10 Mouse epididymal fat pads were dissected out, minced into degradation buffer (Krebs-Ringer 25 mM HEPES, 4% HSA, 1.1 mM glucose, 0.4 mg/ml Collagenase Type 1, pH 7.4), and degraded for up to 1.5 h at 36.5°C. After filtration, washing (Krebs-Ringer HEPES, 1% HSA) and resuspension in assay buffer (Krebs-Ringer HEPES, 1% HSA), cells were
- 15 pipetted into 96-well Picoplates (Packard), containing test solution and approximately an ED_{20} insulin. The assay was started by addition of ^3H glucose (Amersham TRK 239), in a final concentration of 0.45 mM glucose. The assay was incubated for 2 h, 36.5°C, in a Labshaker incubation tower, 400 rpm, then terminated by the addition of PermaBlend/Toluene scintillant
- 20 (or equivalent), and the plates sealed, before standing for at least 1 h and detection in a Packard Top Counter or equivalent. A full insulin standard curve (8 dose) was run as control on each plate. Data are presented graphically, as effect of compound on an (approx) ED_{20} insulin response, with data normalized to a full insulin response. The assay can also be run at
- 25 basal or maximal insulin concentration. Representative dose-response curves for insulin and IGF-1 are shown in figures 71A-71Z; 71A2-71Z2; 71A3-B3. Qualitative references are shown in Table 14.

TABLE 14

Comp. 1	Resp. 2	#expts 3	ED ₅₀ 4	Comments 5
D101	0	4		
D102	0	2		Precipitates
D103	0	2		
D104	0	2		Precipitates
D105	0	2		
D106	0	2		Precipitates
D107	-2	2		
D108	-1	2		
D110	-1	2		
D110	-2	4		
D111	0	2		
D112	0	5		Precipitates
D113	+2	7	Approx 20 μ M	Insoluble, especially after freeze-thaw, resulting in inconsistent results. Some response at basal insulin.
D114	0	2		
D115	0	3		
D116	+2	4	> 20 μ M	Slight effect at basal insulin
D117	+2	8	Approx 20 μ M	Precipitates. Under assay conditions, soluble at least up to 20 μ M (no ppt in microscope, low magnification). Some response at basal insulin.
D117K	+2	2	> 20 μ M	
D118	+2	5	Approx 20 μ M	Biphasic dose response curve (needs repeating)
D119	+1	2		
D120	-1	4		
D121	-1	3		
D122	-1	6		
D123	-1	5		Precipitates
D124	0	5		Precipitates
D125	0	2		
D126	0	2		

¹ Includes series "A" e.g. D101A

² Subjective ranking, on a scale of -2 (antagonist) to +2 (agonist)

³ Includes experiments run at basal and sub maximal insulin concentrations

⁴ Estimated, not calculated values.

⁵ "Precipitates" indicates precipitate in diluted stock prior
to adding to assay. May be soluble under assay conditions

Results:

The binding assays showed that most of the peptides completely inhibited insulin binding to HIR with IC_{50} -values ranging from 0.3 to 20 μ M.

- 5 One peptide (D124) was active at lower concentration but only displaced insulin partially (see Figure 71). One peptide (D112) had high affinity for HIGF-1R, but all the others showed 2-20 fold selectivity for HIR (see Figure 71).

- 10 In the effect assay (FFC), several of the peptides had no effect, some were antagonists, and a few were agonists reaching a response comparable to that of full insulin stimulation. The ED_{50} for the best peptides (D113 and D117) was around 20-30 μ M.

- 15 Despite a right shifted dose response curve relative to insulin, these peptides represent the first non-insulin compounds ever found to elicit a maximal insulin response by binding to the insulin receptor. Such peptides may be useful for development as therapeutics themselves.

The peptides could also be useful as leads for further characterization of molecular requirements for binding to and activation of IR, and/or as tools for identification of the mechanisms involved in the activation.

- 20 Analysis of affinity and activity of another group of peptides is shown in Table 15. In addition to presenting data on the single chain or looped peptide, Table 15 also reports data showing high affinity binding of certain dimers.

TABLE 15

Name	Sequence	HIR affinity mol/l	FFC
S105	FHENFYDWFVRQVAKK-NH ₂	3.1*10 ⁻⁷	++
S106	FHENFYDWFVRQASKK-NH ₂	4.2*10 ⁻⁷	++
S107	FHENFYDWFVRAVSKK-NH ₂	10.0*10 ⁻⁷	+
S108	FHENFYDWFVAQVSKK-NH ₂	7.5*10 ⁻⁷	+
S109	FHENFYDWFARQVSKK-NH ₂	2.3*10 ⁻⁷	++
S110	FHEAFYDWFVRQVSKK-NH ₂	2.2*10 ⁻⁷	++
S111	FHANFYDWFVRQVSKK-NH ₂	3.3*10 ⁻⁷	0
S112	FAENFYDWFVRQVSKK-NH ₂	6.1*10 ⁻⁷	+
S113	AHENFYDWFVRQVSKK-NH ₂	5.9*10 ⁻⁷	+
S114	fhenfydwfvrqvsck	8.3*10 ⁻⁶	0
S115	EFHENFYDWFVRQVSEE	6.5*10 ⁻⁷	+
S116	FHENFYGWVFRQVSKK	1.4*10 ⁻⁶	++
S117	HETFYSMIRSLAK	2.7*10 ⁻⁶	0
S118	SDGFYNAIELLS	2.4*10 ⁻⁶	+
S119	SLNFYDALQLLAKK	1.8*10 ⁻⁶	0
S120	HDPFYSMMSLLK	2.0*10 ⁻⁶	0
S121	NSFYEALRMLSSK	3.1*10 ⁻⁶	0
S122	HPTSKEIYAKLLK	9.3*10 ⁻⁶	0
S123	HPSTNQMLMKLFLK	1.6*10 ⁻⁵	0
S124	HPPLSELKFLIKK	2.3*10 ⁻⁵	0
S125	HAPLSVLVQALLKK		0
S126	HPSLSDMRWILLK		
S127	WSDFYSYFQGLD	1.2*10 ⁻⁶	0
S128	D117-Dap(D117)	1.1*10 ⁻⁶	++
S129	SSNFYQALMLLS	2.9*10 ⁻⁶	0
S131	D117-Dap(CO-CH ₂ -O-NH ₂)	1.2*10 ⁻⁶	+
S137	HENFYGWVFRQVSKK	7.7*10 ⁻⁷	0
S145	D117-Lys(D117)	1.5*10 ⁻⁶	++
S147	D117-b-Ala-Lys(D117)	9.3*10 ⁻⁷	++
S148	D117-b-Ala-Dap(b-Ala-D117)	1.1*10 ⁻⁶	++
S149	D117-Gly-Lys(Gly-D117)	2.0*10 ⁻⁶	++
S150	D117-b-Ala-Lys(b-Ala-D117)	6.2*10 ⁻⁷	++
S152	D117-Dab(D117)	5.2*10 ⁻⁶	+
S153	D117-Orn(D117)	3.9*10 ⁻⁶	+
S154	D117-Dap(b-Ala-D117)	3.6*10 ⁻⁶	+
S155	D117-b-Ala-Orn(b-Ala-D117)	2.5*10 ⁻⁶	++
S156	1-(Thia-b-Ala-D117) ₂		
S157	FHENFYDWFVRQVS		
S158	FHENFYDWFVRQVSK	8.1*10 ⁻⁷	+
S159	FHENFYDWFVQVSK		

S160	FHENFYDWFVSK		
S161	FHENFYDWFVSK		
S162	FHENFYDWFVK		
S165	FYDWF-NH ₂	$>2*10^{-5}$	0
S166	FYDWFKK-NH ₂	$>2*10^{-5}$	0
S167	AFYDWFakk-NH ₂	$>2*10^{-5}$	(-)
S168	AAAAFYDWFAAAAKK-NH ₂	$3.8*10^{-6}$	0
S169	(D117) ₂₋₁₂	$5.8*10^{-7}$	++
S170	(Cys-Gly-D117) ₂	$7.0*10^{-7}$	+++
S171	Cys-Gly-D117	$2.9*10^{-6}$	+++
S172	(D117) ₂₋₁₄	$4.8*10^{-6}$	+++
S173	LDALDRLMRYFEERPSL-NH ₂	$1.2*10^{-6}$	0
S174	PLAELWAYFEHSEQGRSSAH-NH ₂	$1.6*10^{-5}$	0
S175	GRVDWLQRNANFYDWFVAELG-NH ₂	$2.3*10^{-7}$	+++
S176	NGVERAGTGDNFYDWFVAQLH-NH ₂	$4.7*10^{-7}$	+
S177	EHWNTVDPFYFTLFEWLRESG-NH ₂	$2.7*10^{-6}$	0
S178	EHWNTVDPFYQYFSELLRESG-NH ₂	$1.3*10^{-7}$	++
S179	QSDSGTVHDRFYGWFRDTWAS-NH ₂	$5.4*10^{-7}$	+
S180	AFYDWFak-NH ₂	$>2*10^{-5}$	0
S181	AFYDWFa-NH ₂	$>2*10^{-5}$	0
S182	AFYDWF-NH ₂	$>2*10^{-5}$	0
S183	FYDWDA-NH ₂	$>2*10^{-5}$	0
S184	Ac-FYDWF-NH ₂	$>2*10^{-5}$	0
S203	Lig-FHENFYDWFVRQVSKK		
S204	Lig-GGGFHENFYDWFVRQVSKK		
S205	FHENFYDWFVRQVSKKGGG-Lig		
S206	Lig-CAWPTYWNCG		
S207	ACAWPTYWNCG-Lig		
S208	ACAWPTYWNCGGGG-Lig		
S209	Lig-SDGFYNAIELLS		
S210	SDGFYNAIELLS-Lig		
S211	SDGFYNAIELLSGGG-Lig		
S212	KHLCVLEELFWGASLFGYCSGKK-Lig		
S213	AFYDWFakk-Lig		
S214	AFYEWFAKK-NH ₂	$>2*10^{-5}$	0
S215	AFYGWFAKK-NH ₂	$>2*10^{-5}$	0
S216	AFYKWFAKK-NH ₂	$>2*10^{-5}$	0
S217	(SDGFYNAIELLS-Lig) ₂₋₁₄	$3.9*10^{-8}$	++
S218	(AFYDWFakk-Lig) ₂₋₁₄	$1.1*10^{-5}$	0
S219	FHENAYDWFVRQVSKK	$>2*10^{-5}$	0
S220	FHENFADWFVRQVSKK	$>2*10^{-5}$	0
S221	FHENFYAWFVRQVSKK	$1.1*10^{-6}$	(+)
S222	FHENFYDAFVRQVSKK	$>2*10^{-5}$	0

S223	FHENFTDWA VRQVSKK	$>2 \times 10^{-5}$	0
S224	FQSLLEELVWGAPLFRYGTG	$>2 \times 10^{-5}$	0
S225	PLCVLEELFWGASLFGQCSG		
S226	QLEEEWAGVQCEVYGRECPs	1.6×10^{-6}	
S227	Cys-(Gly) ₂ -D117	5.1×10^{-7}	++
S228	(Cys-(Gly) ₂ -D117) ₂	3.6×10^{-7}	++
S229	(S210)-14-(S212)	4.4×10^{-9}	0
S230	(S131)-14-(S212)		
S231	(S205) ₂ -14	2.7×10^{-7}	+
S232	(S204) ₂ -14	3.8×10^{-7}	+++
S233	(S131)-14-(S210)	2.6×10^{-7}	+
S234	RVDWLQRNANFYDWFVAELG	1.3×10^{-7}	++
S235	VDWLQRNANFYDWFVAELG	5.3×10^{-8}	++
S236	DWLQRNANFYDWFVAELG	1.0×10^{-7}	++
S237	WLQRNANFYDWFVAELG	8.5×10^{-7}	0
S238	LQRNANFYDWFVAELG	8.5×10^{-7}	0
S239	QRNANFYDWFVAELG	1.3×10^{-6}	0
S240	RNANFYDWFVAELG	1.4×10^{-6}	
S241	NANFYDWFVAELG	1.6×10^{-6}	
S242	ANFYDWFVAELG	2.0×10^{-6}	
S243	NFYDWFVAELG	2.0×10^{-6}	
S244	GRVDWLQRNANFYDWFVAELG-Lig	2.2×10^{-7}	++
S245	Lig-GRVDWLQRNANFYDWFVAELG	2.2×10^{-7}	+
S246	(S208)-14-(S131)	5.0×10^{-6}	
S247	(S208)-14-(S209)		
S248	GRVDWLQRNANFYDWFVAEL	6.3×10^{-8}	++
S249	GRVDWLQRNANFYDWFVAE	7.4×10^{-7}	0
S250	GRVDWLQRNANFYDWFVA	8.9×10^{-6}	0
S251	GRVDWLQRNANFYDWFV	5.6×10^{-6}	
S252	14-(SDGFYNIAIELLS-Lig) ₂	4.4×10^{-7}	0
S253	(GRVDWLQRNANFYDWFVAELG)-14	2.2×10^{-8}	++
S254	14-(GRVDWLQRNANFYDWFVAE LG)		
S255	(SDGFYNIAIELLSGGG) ₂ -14	1.6×10^{-6}	0
S256	H-Acy-CLEE-w-GASL-Tic-QCSG-NH ₂	9.0×10^{-6}	(-)
S257	RWPNFYGYFESLLTHFS-NH ₂	1.4×10^{-5}	0
S258	HYNAFYEFQVLLAETW-NH ₂		
S259	EGWDFYSYFSGLLASVT-NH ₂	7.7×10^{-6}	0
S260	LDRQFYRYFQDLLVGF-NH ₂	2.3×10^{-6}	0
S261	WGRSFYRYFETLLAQGI-NH ₂	$>2 \times 10^{-5}$	0
S262	PLCFLQELFGGASLGGYCSG-NH ₂	1.9×10^{-5}	0
S263	WLEQERAWIWCEIQSGCRA-NH ₂	$>2 \times 10^{-5}$	0
S264	IQGWPEFYGWFDVV AQMFEE-NH ₂	1.9×10^{-7}	0
S265	TGHRLLGLDEQFYWWFRDALSG-NH ₂	1.1×10^{-7}	0

S266	H- Abu -CLEE- w -GASL-Tic-QCSG-NH ₂	$>2*10^{-5}$	0
S267	14-(Dap-CAWPTYWNCG) ₂		
S268	RDHypFYDWFDDi-NH ₂	$4.5*10^{-7}$	0
S273	S131-14-S209	$1.5*10^{-6}$	+
S274	S294-14-S210		
S275	S295-14-S210		
S276	S294-14-204		
S277	S295-14-S204		
S278	GFREGQRWYWFVAQVT-NH ₂	$>2*10^{-5}$	0
S279	VASGHVLHGQFYRWFDQFALEE-NH ₂		
S280	VGDFCVSHDCFYGWFLRESMQ-NH ₂		
S281	DLRVLCELFGGAYVLGYCSE-NH ₂	$1.1*10^{-5}$	0
S282	HLVSGEELSWWVALLGQWAR-NH ₂	$>2*10^{-5}$	0
S283	APVSTEELRWGALLFGQWAG-NH ₂	$>2*10^{-5}$	0
S284	ALEEEWAWVQVRSIRSLPL-NH ₂	$>2*10^{-5}$	0
S285	WLEHEWAQIQCELYGRGCTY-NH ₂	$8.3*10^{-7}$	
S286	AAVHEQFYDWFADQYEE-NH ₂		
S287	QAPSNFYDWFVREWDEE-NH ₂	$5.9*10^{-6}$	0
S288	QSFYDYIEELLGGEWKK-NH ₂	$4.3*10^{-6}$	0
S289	DPFYQGLWEWLRESGEE-NH ₂	$>2*10^{-5}$	0
S290	(S204) ₂ -7	$9.0*10^{-7}$	++
S291	(S204) ₂ -9	$1.2*10^{-6}$	++++
S292	(S204) ₂ -12	$7.5*10^{-7}$	++
S293	(S204) ₂ -13	$1.2*10^{-7}$	++
S294	DWLQRNANFYDWFVAEL-Lig	$1.3*10^{-7}$	++
S295	Lig-DWLQRNANFYDWFVAEL	$4.8*10^{-7}$	+
S296	(S209) ₂ -9		
S297	(S210) ₂ -9		
S298	LigKHLCLVEELFWGASLFGYCSGKKKK		
S299	KHLCLVEELFWGASLFGYCSGKKKK-Lig		
S300	(S294) ₂ -14	$5.0*10^{-8}$	+++
S301	(S295) ₂ -14	$6.4*10^{-7}$	+
S302	S-D-G-F-Y-N-A-Acy-E-L-L-S		
S303	S-G-P-F-Y-E-E-Acy-E-L-L-W-Aib		
S304	G-G-S-F-Y-D-D-Acy-E-Aib-L-W-Aib		
S305	N-Aib-P-F-Y-D-E-Acy-D-E-Cha-W-Aib		
S306	GRVDWLQRNANFYDWFVAEAcyG-NH ₂		

7, 9, 12, 13, and 14 represent specific chemical linkers (see Table 18)
 FFC: 0 is no effect, + is agonist, - is antagonist

Example 27: Formula 8 synthetic Peptides with Their Affinities for the Human Insulin Receptor (HIR)

A commercial phage display peptide library (New England Biolabs Ph.D.-C7C Disulfide Constrained Peptide Library) was screened for
5 members which bind to IR.

A. Identification of IR Binding Phage

Binding of phage with displayed peptides was detected by ELISA assay. Plates were coated with anti-FC antibody for 2 h at RT or overnight at 4°C. Nonspecific sites were blocked with skim milk (2%) for 1 h at RT.
10 'sIR-FC, a modified form of IR in which the cytoplasmic region is substituted with an IgG-Fc fragment (Bass *et al.*, 1990), was then added to the wells for 2 h at RT. Phage were then added to wells and incubated with or without competing peptides for 2 h at RT. Binding was detected with an anti-phage HRP antibody which was added to the wells and incubated for 2.5 h. at RT.
15 OPD (o-phenylenediamine) color reaction was detected between 5 and 10 min.

B. Characterization of Phage Displayed Peptides

Fifteen different phage were isolated from a linear 12-mer peptide library (New England Biolabs) panned against a dimer of the LI portion of IR
20 (IR Δ703) (Kristensen *et al.*, 1998) Table 16. The displayed sequences were divided into three groups based on their consensus sequences which correspond to Formula motifs 1, 2 and 7. As can be seen in Table 16, the peptides of motif 7 bind strongly to sIR but not sIGF-1R-FC.

The ability of certain peptides identified in the phage library to
25 compete with other peptides is shown in Table 17 below.

J101 (see Figure 8), the peptide expressed by phage CP42, and containing the Formula 8 motif was found to displace insulin from IR with an IC₅₀ of about 5 μm and to be an antagonist in the receptor autophosphorylation and fat cell assays. J101 also does not bind the IR

$\Delta 703$ construct and is not displaced from IR by insulin. Accordingly, J101, may bind IR outside of the insulin binding site. J101, which contains two cysteine residues is likely to have a cyclic structure.

- Phage displaying IR binding peptides were also identified by binding
- 5 phage to plates coated with sIR-Fc as discussed above and washing away non-binding phages. Binding phage were eluted with glycine-HCl, pH 2.2 for 10 min.

The sequences of the displayed peptides which bind IR are shown in Figure 8.

- 10 A few of the peptides (e.g. J101 and J115) (Figure 8) were tested in the fat cell assay and all were full antagonists.

- 125 -

TABLE 16

IM no.	Isolate	Displayed peptide sequence	No. Found	Relative binding to			Formula
				sIR	sIGF ₁ IR-GC	Motif	
IM445	Δ-12.3 #6;	APTFAVENQQT-GGGS	1	+++	+		1
IM447	Δ-12.3 #45;	SFYEAHLQLGV-GGGS	23	+++	+++		2
IM460	Δ-12.3 #76;	NSFYEARLMLSS-GGGS	2	++	(+)		2
IM453	Δ-12.3 #12;	SLNFYDALQLLA-GGGS	1	+++	++		2
IM456	Δ-12.3 #146	SSNFYQALMLLS-GGGS	1	+++	++		2
IM448	Δ-12.3 #40	SDGFYNAIELLS-GGGS	3	+	(+)		2
IM446	Δ-12.3 #24	HETFYSMIRSLA-GGGS	60	+++	+++		2
IM455	Δ-12.3 #10	HDPFYSIMIKSLA-GGGS	1	+++	++		2
IM465	Δ-12.3 #193	WSDPFTSYQQLD-GGGS	1	+++	(+)		2
≥50% Consensus:							
		__FY_AL_L__					
IM452	Δ-12.3 #23;	HPPLEHLKALL-GGGS	4	++++	-		7
IM451	Δ-12.3 #34;	HPPLSELKFLI-GGGS	33	++++	-		7
IM459	Δ-12.3 #60;	HPPLSDMRWILL-GGGS	2	+++	-		7
IM458	Δ-12.3 #30;	HAPLSVLAQALL-GGGS	2	++	++		7
IM449	Δ-12.3 #43;	HPTSKIEVAKLL-GGGS	14	++	-		7
IM450	Δ-12.3 #28;	HPSTINQMLMKLF-GGGS	40	++	-		7
≥50% Consensus:							
		HPPLS__L_LL					
		S122					
		2123					

TABLE 17

Phage	Sequence Formula Motif	D103 1	D118 2	D119 2	D120 10	D121 10	D122 10	D123 6	D124 4	Insulin
IM332(-101)	Cyclic	% 85	% 100	% 100	% 100	% 100	% 100	% 100	+71	% 98
IM445(-1229)	APTEYAWFNQDT	++0	++2	++0	++0	++0	% 100	% 100	+68	++11
IM447(-1227)	SPYEAHQLLSV	++0	++0	++0	++0	++0	% 85	+58	+46	++3
IM452(-1228)	HPREHLKAFLL	++0	++0	++10	++0	++0	% 95	nd	% 84	+26
IM242(-1F)	LPI	+37	++17	+46	+30	+66	+57	+55	++19	++0

% :>80% signal (not displaced)

+: 20-70% signal

++: <20% signal (fully displaced)

EXAMPLE 28: PREPARATION OF THE DIMERS

A. Materials

Generally, suitably protected N-Fmoc (fluorenylmethoxycarbonyl)-amino acids were purchased from Novabiochem (Switzerland), 1-hydroxy-7-azabenzotriazole (HOAt) from Perspective Biosystems and *N,N'*-diisopropylcarbodiimide (DIC) from Fluka. The molecular weights of the peptides were determined using matrix-assisted laser desorption time-of-flight mass spectroscopy (MALDI-MS), recorded on a Voyager-DE (Perseptive Biosystems). A matrix of sinapinic acid was used. Analytical and semi-preparative high-pressure liquid chromatography (HPLC) were performed using a Waters RCM 8 x 10 module and with a C-18 column (19 x 300 mm) and a C-18 column (25 x 300 mm), respectively, at 40°C. The solvent system for both analytical and semi-preparative HPLC was buffer A; 0.1% TFA in water and buffer B; 0.07% TFA in 100% and UV detection was at 215 nm. The gradient for analytical HPLC (1.5 ml/min); a linear gradient of 5-90% buffer B over 25 min and semi-preparative HPLC (4 ml/min); an isocratic gradient of 20% buffer B over 5 min, followed by a linear gradient of 20-60% buffer B over 40 min.

B. Solid-Phase Peptide Synthesis and Analysis of the D117 Monomer(FHENFYDWFVRQVSKK-Dap(CO-CH2-O-NH2))

The peptide monomer available for ligation was synthesized manually in plastic syringes using a preloaded Rink amide linker (RAM)-TentaGel (0.26 mmol/g). Fully protected N-Fmoc amino acids (3 equiv.) were used and the temporary Fmoc protecting group was removed after each cycle by 30% piperidine in *N*-methylpyrrolidone (NMP). The natural amino acids were coupled as their free acids in NMP using DIC (3 mol equiv.) and HOAt (3 mol equiv.) as coupling additive.

First, Fmoc-Dap(Alloc) was coupled as described above. The alloc group was then removed by Pd(0) (3 mol equiv.) in CHCl₃/AcOH/N-


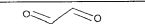
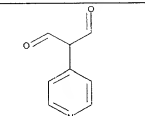
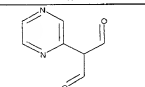

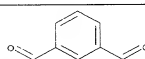

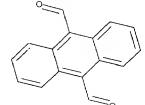
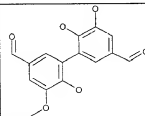
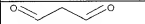
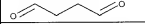

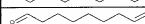

methylmorpholine (37:2:1, v/v/v) under helium. After 2 h. at RT, the resin was washed with 5% in NMP containing 2% diethyldithiocarbamide, Na salt. Finally, the resin was washed with NMP containing HOBt (hydroxybenzotriazole). The protected oxyamino acetic acid (3 mol equiv.)

- 5 was then coupled on the side-chain of Dap (diaminopropionic acid). The completion of all the acylation reactions was monitored visually by the use of bromophenol blue. Between the Fmoc-deprotection and the acylation reaction, the resin was washed with NMP (x 6).

- After synthesis, the peptide was washed with DCM (dichloromethane) (x 3). The peptides were cleaved simultaneously from the resin and the side-chain protecting groups were removed by treatment with 95% aqueous TFA containing triisopropylsilan (TIS) (4 molar equiv.) for 1.5 h. The resin was rinsed with 95% aqueous acetic acid (x 4). Both TFA and acetic acid were evaporated and the peptide was finally precipitated in diethyl ether and lyophilized overnight. The peptide was both analyzed by analytical HPLC and MALDI-MS. Analysis by MALDI-MS; m/z 2287.5 (M + H)⁺ (requires m/z , 2288.3) confirmed the expected product.
- 10
15

- To the peptide monomer, FHENFYDWFVRQVSKK-Dap(CO-CH₂-O-NH₂) (9.1 mg, 3.9 mol) was added the dialdehyde linker (0.81 mol) dissolved in 80% DMSO (aqueous) (28 l). The pH was then adjusted to 5 with solid sodium acetate. The solution was left overnight at 37°C and progress of the reaction was monitored by RP-HPLC. The formed dimer (see Table 18) was purified by semi-preparative HPLC. Analysis by MALDI-MS confirmed the expected product (see Table 18). The molecular weights and inter peptide distance of various linkers is shown below.
- 20
25

TABLE 18

Structure	Number	MW	MW (- 2H ₂ O)
	<u>1</u>	100.1	64.1
	<u>2</u>	58.04	22.04
	<u>3</u>	149.15	113.15
	<u>4</u>	150.14	114.14
	<u>5</u>	134.13	98.13
	<u>6</u>	134.13	98.13
	<u>7</u>	134.13	98.13
	<u>8</u>	234.25	198.25
	<u>9</u>	302.3	266.3
	<u>10</u>	72.06	36.06
	<u>11</u>	86.09	50.09
	<u>12</u>	114.14	78.14
	<u>13</u>	128.08	92.08
	<u>14</u>	142.19	106.19

Dimers were prepared by ligation chemistry (oxime bond in the ligation site) (attached through C-terminal domain).

C. Binding of Dimers to Different IR Constructs Indicates Peptides Bind to Two Independent Sites

5 Table 19 summarizes the results of binding of phages of D117 (Formula 1 Motif), D123 (Formula 6 motif), D124 (Formula 4 motif), and CP42 (phage expressing peptide J101, Formula 8 motif) monomer to constructs of IR consisting of the L1-cys-L2 region, L1-cys-L2-FnIII α region and L2-FnIII α region.

TABLE 19

IR Construct	Peptides Bound	Motif
L1-cys-L2	D117	A6 Only
L1-cys-L2-FnIII α	D117, CP42, D123, 124	A6, D8, F8
L2-FnIII α	CP42, D123, D124	D8, F8

15 The data above is consistent with a conclusion that the A6 (Formula 1 motif) and F8 (Formula 4 Motif) motifs are physically distinct and on separate parts of IR. Competition data, supra, further indicates that the binding site for the B6 (Formula 2 motif) is on the same subunit as that for the A6 motif.

As shown below, BIAcore competition studies are consistent with the separation of Sites 1 (A6, B6) and 2 (D8, F8, J101).

20 **D. Competition of Site 1 and Site 2 Phage Displayed Peptides with Recombinant Cleaved Di-Peptides**

Insulin receptor was coated on a 96-well plate with 50 μ l of a 2 ng/ μ l solution of IR and incubated overnight at 4°C. The wells were then blocked with MPBS for 1 h.

Dimers were prepared by expressing them as MBP fusion products. See, Table 1, supra. The sequences of the MBP- cleaved dimers are shown below (core peptide sequences are underlined):

Cleaved Dimer Sequences

- 5 **#426 (D8)**
AQPAMAWLDQEWAWVQCEVYGRGCPSSAAAGAPVPYPDPLEPRAA.
- #429(D8-6-D8)**
AQPAMAWLDQEWAWVQCEVYGRGCPSSGGSGGSLDQEWAWVQCEVY
10 GRGCPSSAAAGAPVPYPDPLEPRAA.
- #459 (short flag RB6)**
ISEFGSADYKDLDALDRLMRYFEERPSLAAAGAPVPYPDPLEPRAA.
- 15 **#430 (H2C-4-RB6)**
DYKDDDDKFHENFYDWVFRQVSGGSLDALDRLMRYFEERPSLAAAGAP
VPYPDPLEPRAA.
- #464 (H2C)**
20 DYKDDDDFHENFYDWVFRQVSAAAGAPVPYPDPLEPRAA.
- #446 (F8)**
DYKDDDDHLCVLEELFWGASLFGYCSGAAAGAPVPYPDPLEPRAA.
- 25 **#431 (H2C-6-F8)**
DYKDDDDKFHENFYDWVFRQVSGGSGGSHLCVLEELFWGASLFGYCSC
AAAGAPVPYPDPLEPRAA.
- #433 (H2C-9-F8)**
30 DYKDDDDKFHENFYDWVFRQVSGGSGGSGGSHLCVLEELFWGASLFGY
CSGAAAGAPVPYPDPLEPRAA.
- #432 (H2C-12-F8)**
DYKDDDDKFHENFYDWVFRQVSGGSGGSGGSGGSHLCVLEELFWGASL
35 FGYCSCGAAAGAPVPYPDPLEPRAA.
- #452 (G3)**
AQPAMARGGGTFYEWFEALRKHGAGAAAGAPVPYPDPLEPRAA.
- 40 **#427 (G3-6-G3)**
AQPAMARGGGTFYEWFEALRKHGAGGGSGGSRGGGTFYEWFEALRK
HGAGAAAGAPVPYPDPLEPRAA.
(* A TO T CHANGE)

- #428 (G3-12-G3)**
AQPAMARGGGTFYEWFESALRKHGAGGGSGGSGGSGGSRGGGTFYEW
FESALRKHGAGAAAGAPVPYPDPLEPRAALTN.
- 5
- #434 (G3-12-G3)**
ISEFIEVRAQPAMARGGGTFYEWFESALRKHGAGGGSGGSGGSGGSRG
GGTFYEWFESALRKHGAGAAAGAPVPYPDPLEPRAA.
- 10
- #437 (H2C)**
AQPAMAFHENFYDWFVRQVSAAGAPVPYPDPLEPRAA.
- 15
- #463 (H2C-3-H2C)**
AQPAMAFHENFYDWFVRQVSGGSFHENFYDWFVRQVSAAGAPVPYPD
PLEPRAA.
- #435 (H2C-3-H2C-3-H2C)**
AQPAMAFHENFYDWFVRQVSGGSFHENFYDWFVRQVSGGSFHENFYD
WFVRQVSAAGAPVPYPDPLEPRAA.
- 20
- #439 (H2C-6-H2C)**
AQPAMAFHENFYDWFVRQVSGGSGGSFHENFYDWFVRQVSAAGAPVP
YPDPLEPRAA.
- 25
- #436 (H2C-9-H2C)**
AQPAMAFHENFYDWFVRQVSGGSGGSGGSFHENFYDWFVRQVSAAG
APVPYPDPLEPRAA.
- 30
- #449 (H2C-12-H2C)**
AQPAMAFHENFYDWFVRQVSGGSGGSGGSGGSFHENFYDWFVRQVS
AAAGAPVPYPDPLEPRAA.
- 35
- MBP***
ISEFGSSRVDLQASLALAVVLQRRDWDENPGVTQLNRLAAHPPFASWRNSEE
ARTDRPSQQLRSLNGEWQLGCGFG

The MBP- cleaved fusion protein mixtures were appropriately diluted, added to the wells, and incubated at RT for 30 min. An equal volume of F8 or H2C phage displayed peptide was then added to each well and incubated for 1 h. The control wells (100% phage binding) contained only phage and an equal volume of buffer. The control cleaved fusion protein mixture contains a peptide derived from the lacZ gene. The plate was washed 3

times in PBST and then incubated with HRP/anti-M13 conjugate for 45 min. The plate was washed again and then the ABTS substrate added. The values indicate readings taken at OD₄₀₅. Figure 72A shows competition between cleaved monomers and dimers and F7 phage for binding to Site 2 of IR. Figure 72B shows competition for binding to Site 1 between H2C and the cleaved and uncleaved monomers and dimers. IC₅₀ values are shown in Table 20.

TABLE 20

Dimers Site 1/Site 2 IC ₅₀ Values				
Phage Signal	H2C		F8	
Cleavage	-	+	-	+
Dimers				
LF-H2C(6)F8	0.2	0.19	0.3	5
LF-H2C(9)F8	0.4	0.11	3	15
LF-H2C(12)F8	0.3	0.19	>16	16
LF-F8 mono	-	-	>20	12
LF-H2C mono	0.145	0.11	>1	>1
H2C mono	0.3	0.2	>0.5	>0.5
MBP-lacZ control	-	-	-	-

- = uncleaved
+ = cleaved

E. Stimulation of autophosphorylation of IR by MBP-Fusion Peptides

Fusion peptides were prepared as described above, and then assayed for IR activation (see Example 20). The results of these experiments shown in Figure 74 indicate that the H2C monomer and H2C-H2C homodimers stimulate autophosphorylation of IR *in vivo*.

H2C dimers (Site 1-Site 1) with a 6 amino acid linker (H2C-6-H2C) were most active in the autophosphorylation assay. Other active dimers are also shown in Figure 74, particularly H2C-9-H2C, H2C-12-H2C, H2C-3-H2C, and F8.

Example 29: IGF-1R Peptide Assays

A. IC₅₀ Determinations

Peptides that meet the proper criteria of affinity, selectivity, and activity may be used to develop site-directed assays to identify active molecules which bind to sites on IGF-1R. Assays have been developed using Time-Resolved Fluorescence Resonance Energy Transfer (FRET). These assays are not radioactive, homogeneous (no wash steps), and can be rapidly carried out in 96- or 384-well microtiter plate format facilitating their use in high-throughput screening assays for small organic molecules.

This assay can be used to assess the affinity of peptides for IGF-1R or can be used to find small organic molecule leads in a high-throughput capacity. The determination of the IC₅₀ for several peptides is described below.

1. Assay Components

IGF-1R was purchased from R&D System, Cat. # 391-GR/CF. The IGF-1R was labeled with Europium (Eu) by EG&G Wallac. Ten milligrams of IGF-1R was sent to Wallac and the IGF-1R was labeled with Wallac's W-1024 Eu-chelate.

The Streptavidin-Allophycocyanin (SA-APC) was obtained from Prozyme Cat. # PJ25S. The biotinylated 20E2 [DYKDFYDAIDQLVRGSARAGGTRDKK(ε-biotin)] ("b-20E2") was synthesized by Novo Nordisk or by PeptidoGenic Research & Co., Inc. The IGF-1 was commercially available from PeproTech Cat. # 100-11.

2. Assay Method

a. Preparation of the Assay Mix. A 2X concentration of Assay Mix consisting of 4 nM Eu-labeled IGF-1R, 30 nM b-20E2, 4 nM SA-APC, and 0.1% BSA was prepared. This mixture was allowed to pre-incubate at RT in the dark for 1-2 h before competitor was added.

b. Dilutions of the Competitors were carried out on a 96-well microtiter plate (Costar Cat. #3912). 100 μ l of Buffer (TBS pH 8.0 + 0.1 % BSA) were dispensed to wells in columns 1 through 11. Competitors and Buffer were added to Column 12 wells so that the total volume is 150 μ l.

5 c. To identify small organic compounds which also bind the active sites of IGF-1R, dilutions of the small organic compounds are also performed on a 96-well microtiter plate (Costar Cat. #3912). Compounds are dissolved in 100% DMSO. Therefore, 100 μ l of Buffer (TBS pH 8.0 + 0.1 % BSA) with 4% DMSO are dispensed to wells in columns 1 through 10.
10 Column 11 contains 100 μ l of Buffer with 2.7% DMSO. Compounds (6 μ l) are added into 144 μ l Buffer (No DMSO) to Column 12 wells.

d. Dilutions were performed across columns on the plate. Once competitors were dispensed into Column 12 and mixed, 50 μ l of the solution Column 12 were transferred to wells in 11 and mixed. 50 μ l of the Column
15 11 mixture was transferred to Column 10 wells. This was repeated until 50 μ l of Column 3 mixture was transferred to Column 2 wells. Once accomplished to Column 2, 50 μ l from Column 2 were removed and discarded. Column 1 wells were reserved for No Competitor Wells. 100 μ l volume was therefore maintained across all columns.

20 e. 50 μ l of the Assay Mix was dispensed into wells on a new 96-well microtiter plate. 50 μ l from the Dilutions Plate were then added to this plate.

f. 30 μ l from Assay Mix Plate were transferred from the 96-well in duplicate on a 384-well microtiter plate (Nunc Cat. # 264512). This covered
25 plate was allowed to incubate at RT overnight.

g. Binding was measured using Wallac's Victor II fluorometer by excitation at 340 nm and measuring emission at 665 and 615 nm.

h. The working concentrations of this assay were 2 nM Eu-labeled IGF-1R, 15 nM b-20E2, 2 nM SA-APC, and 0.1% BSA. Peptides
30 were normally diluted starting from 100 μ M, where IGF-1 begins at 30 μ M.

Compounds begin at 200 μ M in a working concentration of 2% DMSO.
Controls also contained 2% DMSO.

3. Results

The IC₅₀ and holoenzyme phosphorylation activity (see Example 20)
5 values for certain peptides are shown below.

Peptide Data

<u>Name</u>	<u>Sequence</u>	<u>IGF-1R IC₅₀</u>	<u>Holo. Phos.</u>
IGF-1	Natural Ligand	~1-10 nM	
C1	A6S-4-C1-IGFR or D112	~10 nM	
RP9	H2C Design	33 nM	++
20E2	R20a-3-20E2-IR or D118	~100 nM	
G8	20E2B-3-C6-IGFR	139 nM	-
RP2	H2CB-3-B9-IR	163 nM	+
E8	R20b-4-E8-IR or D120	175 nM	
G33	H2CA-4-G9-IGFR	178 nM	+++
RP6	20C-4-G3-IGFR	184 nM	+++++
RP14	H2CA-4-H8-IGFR	225 nM	
S178	B6C-3-C10-IR	240 nM	
RP10	20E2 Design	315 nM	+
S176	A6S-4-G1-IR	418 nM	
H2C	A6S-4-H2-IGFR or D117	~600 nM	+
B6	R40-3-B6-IGFR	631 nM	
RP13	H2CA-4-H6-IGFR	818 nM	
G8	20E2B-3-C6-IGFR	1330 nM	-
S174	R20-4-F9-IGFR	1460 nM	
RP8	20E2 Design	1800 nM	+
S177	B6C-3-C7-IR	2040 nM	
S175	A6S-3-E12-IR	2050 nM	++
RP1	H2CB-4-G11-IR	2790 nM	+
bS175	A6S-3-E12-IR	3230 nM	
NG C2	20E2-3-C2-IGFR	4020 nM	
S179	H2CBa-3-B12-IR	5350 nM	

S173	rB6-4-A12-IR	5620 nM	
RP5	20E2B-3-B3-IR	7450 nM	-
G9	20E2B-1-A6-IGFR	7550 nM	-
RP4	20E2A-4-F9-IR	8110 nM	+
D8 (B12)	D820-4-B12-IR	11300 nM	
RP24	R20b-4-A4-IR	17800 nM	
RP11	A6S Design	18800 nM	+
D8	R20b-4-D8-IR	21650 nM	
A6	R40-3-A6-IGFR	46600 nM	
RP17	R20b-4-A6-IR	50000 nM	
S167	Short A6	~100 μ M	
RP3	20E2A-3-B11-IR	~100 μ M	-
KC F9	D820-4-F9-IR	~100 μ M	
JB3	CONTROL	~100 μ M	
KC G1	D820-4-F10-IGFR	~100 μ M	
C3-MDM2	CONTROL	>100 μ M	
RP21	40F-4-C1-IGFR	>100 μ M	
RP22	40F-4-D10-IGFR	>100 μ M	
RP23	40F-4-C1-IR	>100 μ M	
KC G2	D820-4-F10-IGFR	>100 μ M	
KC G7	F815-4-G7-IGFR	>100 μ M	

B. IGF-1R Peptide Assay Competition Dissociation

- A competition dissociation experiment was performed to determine if any peptides altered the dissociation rate of the 20E2 (B6 motif) peptide in the IGF-1R Peptide Assay. An alteration of the dissociation rate suggests the peptide used in the competition binds to a second site on IGF-1R thus enhancing or slowing the 20E2 dissociation rate through an allosteric interaction.

1. Materials

- IGF-1R was purchased from R&D System, Cat. # 391-GR/CF. The IGF-1R was labeled with Europium (Eu) by EG&G Wallac. Ten milligrams of

IGF-1R was sent to Wallac and the IGF-1R was labeled with Wallac's W-1024 Eu-chelate.

The Streptavidin-Allophycocyanin (SA-APC) obtained from Prozyme Cat. # PJ25S. The biotinylated 20E2

- 5 [DYKDFYDAIDQLVRGSARAGGTRDKK(ϵ -biotin)] was synthesized by Novo Nordisk or by PeptidoGenic Research & Co., Inc. The IGF-1 was commercially available from PeproTech Cat. # 100-11.

2. Methods

- a. Preparation of the Assay Mix. A 1.25X concentration of Assay
10 Mix consisting of 2.5 nM Eu-labeled IGF-1R, 18.75 nM b-20E2, 2.5 nM SA-APC, and 0.1% BSA was prepared. This mixture was allowed to pre-incubate.

b. 20 μ l of Competitor and Buffer were added to a 96-well microtiter plate (Costar Cat. #3912).

- 15 c. Wallac Victor II Fluorometer was readied to read at 665 nm only in multiple repeats (99) of only the wells containing material.

d. 80 μ l of the 1.25X Assay Mix was added to the 96-well microtiter plate and promptly placed onto the Victor II for readings.

- e. After the original 99 repeat readings were taken, periodic
20 readings were taken until equilibrium had been established.

NOTE: Different conditions can be used for these experiments. For example, a 1.1X concentration of assay mix can be initially made. Then first add 10 μ l of Competitor and Buffer to the microtiter plate followed by 90 μ l of the Assay Mix.

- 25 f. The working concentrations of this assay were 2 nM Eu-labeled IGF-1R, 15 nM b-20E2, 2 nM SA-APC, and 0.1% BSA. Peptides were normally competed at 100 μ M, whereas IGF-1 was competed at 30 μ M. Results are shown in Figure 14.

3. Results

Figure 14 shows the results of one of the experiments. Clearly, IGF-1 and D8 (B12) cause a much slower dissociation rate than the 20E2 (motif 2), H2C (motif 1), C1 (motif 1), and RP6 (motif 2) peptides. This suggests that
5 IGF-1 and D8 (B12) contact IGF-1R in different locations than that of 20E2, H2C, C1, and RP6.

Previous data (EXAMPLE 28) suggests that the motif 6 series binds to a location of IGF-1R that differs from motifs 1 and 2 and that these two sites are not independent of one another. The slowing of the dissociation
10 rate by IGF-1 and D8 (B12) further suggests that there are at least two sites of binding to IGF-1R and that these two sites are not independent of one another.

The following publications, some of which have been cited herein, are
15 cited for general background information and are incorporated by reference in their entirety.

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522815_1

We claim:

1. A method of modulating insulin activity in mammalian cells, said method comprising administering to said cells an amino acid sequence which binds IR and comprises the amino acid sequence $X_1X_2X_3X_4X_5$, wherein X_1 , X_2 ,
5 X_4 , and X_5 are aromatic amino acids, and X_3 is any polar amino acid.
2. The method according to claim 1 wherein X_1 , X_2 , and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptophan, tyrosine and phenylalanine.
- 10 3. The method according to claim 2 wherein said amino acid sequence is an insulin agonist.
4. The method according to claim 2 wherein said amino acid sequence is an insulin antagonist.
5. The method according to claim either one of claims 3 or 4 wherein X_1 and
15 X_5 are phenylalanine and X_2 is tyrosine.
6. The method according to claim 5 wherein X_4 is tryptophan.
7. The method according to claim 6 wherein the amino acid sequence is an insulin agonist and X_3 is selected from the group consisting of aspartic acid and glutamic acid.
- 20 8. The method according to claim 7 wherein X_3 is aspartic acid to result in an amino acid sequence comprising FYDWF.

9. The method according to claim 7 wherein X_3 is glutamic acid to result in an amino acid sequence comprising FYEWF.
10. The method according to claim 1 wherein the amino acid sequence FHEN is bound to the amino terminal of $X_1X_2X_3X_4X_5$ to produce an amino acid sequence comprising FHEN $X_1X_2X_3X_4X_5$ and possessing insulin agonist activity.
11. The method according to claim 10 wherein the amino acid sequence is FHENFYDWF.
12. The method according to claim 1 wherein the amino acid sequence $X_1X_2X_3X_4X_5$ further comprises the amino acid sequence $X_{93}X_{94}X_{95}X_{96}X_{97}$ located at the carboxy terminal end adjacent to X_5 , wherein X_{93} , X_{94} and X_{97} may be any amino acid, X_{95} is selected from the group consisting of glutamine, glutamic acid, alanine and lysine, and X_{96} is a hydrophobic or aliphatic amino acid.
13. The method according to claim 12 wherein X_{93} is selected from the group consisting of alanine, aspartic acid, glutamic acid, arginine, and valine, X_{95} is glutamine or glutamic acid, and X_{96} is selected from the group consisting of leucine, isoleucine, valine and tryptophan.
14. The method according to claim 13 wherein X_{96} is leucine or tryptophan.
15. The method according to claim 14 wherein X_{96} is leucine.
16. The method according to claim 13 wherein X_{95} is glutamine or glutamic acid, and X_{96} is tryptophan.

17. The method according to claim 13 wherein X_{95} is glutamic acid and the amino acid sequence is an insulin agonist.
18. The method according to claim 13 wherein asparagine is present as the amino acid bound to the amino terminal of X_1 and wherein $X_1X_2X_3X_4X_5X_{93}$ is FYDWFV
19. The method according to claim 1 wherein the amino acid sequence is selected from the group of amino acid sequences listed in Figures 1, 2, and 9.
20. The method according to claim 1 wherein the sequence is selected from the group consisting of FHENFYDWFVRQVSK, DYKDVTFSTSAVFHENFYDWFVRQVSKK, GRVDWLQRNANFYDWFVAELG and APTFYAWFNQQT.
21. The method according to claim 1 wherein the sequence is selected from the group consisting of
- FHENFYDWFVRQVAKK-NH₂
FHENFYDWFVRQASKK-NH₂
FHENFYDWFVRAVSKK-NH₂
FHENFYDWFVAQVSCK-NH₂
FHENFYDWFARQVSCK-NH₂
FHEAFYDWFVRQVSCK-NH₂
FHANFYDWFVRQVSCK-NH₂
FAENFYDWFVRQVSCK-NH₂
AHENFYDWFVRQVSCK-NH₂
fhenfydwfvrvqvsck
EFHENFYDWFVRQVSEE
FHENFYGWVVRQVSCK
HETFYSMIRSLAK
SDGFYNAIELLS
SLNFYDALQLLAKK
HDPFYMMKSLK

NSFYEALRMLSSK
HPTSKEIYAKLLK
HPSTNQMLMKLFK
5 HPPLSELKFLIKK
HAPLSVLVQALLKK
HPSLSDMRWILLK
WSDFYSYFQGLD
D117-Dap(D117)
SSNFYQALMLLS
10 D117-Dap(CO-CH₂-O-NH₂)
HENFYGWVFRQVSKK
D117-Lys(D117)
D117-b-Ala-Lys(D117)
D117-b-Ala-Dap(b-Ala-D117)
15 D117-Gly-Lys(Gly-D117)
D117-b-Ala-Lys(b-Ala-D117)
D117-Dab(D117)
D117-Orn(D117)
D117-Dap(b-Ala-D117)
20 D117-b-Ala-Orn(b-Ala-D117)
1-(Thia-b-Ala-D117)₂
FHENFYDWFVRQVS
FHENFYDWFVRQVSK
FHENFYDWFVQVSK
25 FHENFYDWFVVS
FHENFYDWFVSK
FHENFYDWFVK
FYDWF-NH₂
FYDWFKK-NH₂
30 AFYDWFACK-NH₂
AAAAFYDWFAAAAAKK-NH₂
(D117)₂-12
(Cys-Gly-D117)₂
Cys-Gly-D117
35 (D117)₂-14
LDALDRLMRYFEERPSL-NH₂
PLAELWAYFEHSEQGRSSAH-NH₂
GRVDWLQRNANFYDWFVAELG-NH₂
NGVERAGTGDNFYDWFVAQLH-NH₂
40 EHWNTVDPFYFTLFEWLRESG-NH₂
EHWNTVDPFYQYFSELLRESG-NH₂
QSDSGTVHDRFYGWFRDTWAS-NH₂
AFYDWFACK-NH₂

AFYDWFA-NH₂
 AFYDWF-NH₂
 FYDWDA-NH₂
 Ac-FYDWF-NH₂
 5 Lig-FHENFYDWFVRQVSKK
 Lig-GGGFHENFYDWFVRQVSKK
 FHENFYDWFVRQVSKKGGG-Lig
 Lig-CAWPTYWNCG
 ACAWPTYWNCG-Lig
 10 ACAWPTYWNCGGGG-Lig
 Lig-SDGFYNAIELLS
 SDGFYNAIELLS-Lig
 SDGFYNAIELLSGGG-Lig
 KHLCVLEELFWGASLFGYCSGKK-Lig
 15 AFYDWFACK-Lig
 AFYEWFAKK-NH₂
 AFYGWFAKK-NH₂
 AFYKWFAKK-NH₂
 (SDGFYNAIELLS-Lig)₂₋₁₄
 20 (AFYDWFACK-Lig)₂₋₁₄
 FHENAYDWFVRQVSKK
 FHENFADWFVRQVSKK
 FHENFYAWFVRQVSKK
 FHENFYDAFVRQVSKK
 25 FHENFTDWA VRQVSKK
 FQSLLEELVWGAPLFRYGTG
 PLCVLEELFWGASLFGQCSG
 QLEEEWAGVQCEVYGRECP
 Cys-(Gly)₂-D117
 30 (Cys-(Gly)₂-D117)₂
 (S210)-14-(S212)
 (S131)-14-(S212)
 (S205)₂₋₁₄
 (S204)₂₋₁₄
 35 (S131)-14-(S210)
 RVDWLQRNANFYDWFVAELG
 VDWLQRNANFYDWFVAELG
 DWLQRNANFYDWFVAELG
 WLQRNANFYDWFVAELG
 40 LQRNANFYDWFVAELG
 QRNANFYDWFVAELG
 RNANFYDWFVAELG
 NANFYDWFVAELG

ANFYDWFVAELG
 NFYDWFVAELG
 GRVDWLQRNANFYDWFVAELG-Lig
 Lig-GRVDWLQRNANFYDWFVAELG
 5 (S208)-14-(S131)
 (S208)-14-(S209)
 GRVDWLQRNANFYDWFVAEL
 GRVDWLQRNANFYDWFVAE
 GRVDWLQRNANFYDWFVA
 10 GRVDWLQRNANFYDWFV
 14-(SDGFYNAIELLS-Lig)₂
 (GRVDWLQRNANFYDWFVAELG)-14
 14-(GRVDWLQRNANFYDWFVAELG)
 (SDGFYNAIELLSGGG)₂-14
 15 H-Acy-CLEE-w-GASL-Tic-QCSG-NH₂
 RWPNFYGYFESLLTHFS-NH₂
 HYNAFYEYFQVLLAETW-NH₂
 EGWDFYSYFSGLLASVT-NH₂
 LDRQFYRYFQDLLVGFM-NH₂
 20 WGRSFYRYFETLLAQGI-NH₂
 PLCFLQELFGGASLGGYCSG-NH₂
 WLEQERAWIWCEIQSGGCRA-NH₂
 IQGWEPFYGWFDVV AQMFEE-NH₂
 TGHRLGLDEQFYWWFRDALSG-NH₂
 25 H-Abu-CLEE-w-GASL-Tic-QCSG-NH₂
 14-(Dap-CAWPTYWNCG)₂
 RDHypFYDWFDDi-NH₂
 S131-14-S209
 S294-14-S210
 30 S295-14-S210
 S294-14-204
 S295-14-S204
 GFREGQRWYWFVAQVT-NH₂
 VASGHVHLHGQFYRWFDQFALEE-NH₂
 35 VGDFCVSHDCFYGWFLRESMQ-NH₂
 DLRVLCELFGGAYVLGYCSE-NH₂
 HLSVGEELSWVVALLGQWAR-NH₂
 APVSTEELRWGALLFGQWAG-NH₂
 ALEEEWAWVQVRSIRSGPL-NH₂
 40 WLEHEWAQICELYGRGCTY-NH₂
 AAVHEQFYDWFADQYEE-NH₂
 QAPSNFYDWFVREWDEE-NH₂
 QSFYDYIEELGGGEWKK-NH₂

- DPFYQGLWEWLRESGEE-NH₂
 (S204)₂-7
 (S204)₂-9
 (S204)₂-12
 5 (S204)₂-13
 DWLQRNANFYDWFVAEL-Lig
 Lig-DWLQRNANFYDWFVAEL
 (S209)₂-9
 (S210)₂-9
 10 LigKHLCLVLEELFWGASLFGYCSGKKKK
 KHLCLVLEELFWGASLFGYCSGKKKK-Lig
 (S294)₂-14
 (S295)₂-14
 S-D-G-F-Y-N-A-Acy-E-L-L-S
 15 S-G-P-F-Y-E-E-Acy-E-L-L-W-Aib
 G-G-S-F-Y-D-D-Acy-E-Aib-L-W-Aib
 N-Aib-P-F-Y-D-E-Acy-D-E-Cha-W-Aib
 GRVDWLQRNANFYDWFVAEAcyG-NH₂
 and wherein underlined numbers represent a linker as defined in Table 18.
- 20 22. The method according to claim 2 wherein the amino acid sequence binds to
 the insulin receptor with an affinity of at least about 10⁻⁵ M.
23. The method according to claim 22 wherein the affinity is at least about 10⁻⁷
 M.
24. The method according to claim 23 wherein the affinity is at least about 10⁻⁹
 25 M.
25. An amino acid sequence comprising X₁X₂X₃X₄X₅ wherein X₁, X₂, X₄, and
 X₅ are aromatic amino acids, X₃ is any polar amino acid, and wherein said
 amino acid sequence binds to IGF-1R.
26. The amino acid sequence according to claim 25 wherein the IGF-1R binding
 30 occurs with an affinity (K_d) of at least about 10⁻⁵ M.

27. The amino acid sequence according to claim 25 wherein the binding occurs at an affinity (K_d) of at least about 10^{-7} M.
28. The amino acid sequence according to claim 25 wherein X_1 , X_2 , and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptophan, tyrosine and phenylalanine.
29. The amino acid sequence according to claim 28 wherein X_3 is selected from the group consisting of aspartic acid and glutamic acid.
30. The amino acid sequence according to claim 29 wherein X_1 and X_5 are phenylalanine and X_2 is tyrosine.
31. The amino acid sequence according to claim 29 wherein X_4 is tryptophan.
32. The amino acid sequence according to claim 31 wherein X_3 is aspartic acid to result in an amino acid sequence comprising FYDWF.
33. The amino acid sequence according to claim 31 wherein X_3 is glutamic acid to result in an amino acid sequence comprising FYEWF.
34. The amino acid sequence according to claim 28 wherein the amino acid sequence FHEN is bound to the amino terminal of $X_1X_2X_3X_4X_5$ to produce an amino acid sequence comprising FHEN $X_1X_2X_3X_4X_5$.
35. The amino acid sequence according to claim 34 wherein the amino acid sequence comprises FHENFYDWF.

36. The amino acid sequence according to claim 25 wherein the amino acid sequence $X_1X_2X_3X_4X_5$ further comprises the amino acid sequence $X_{93} X_{94} X_{95} X_{96} X_{97}$ located at the carboxy terminal end adjacent to X_5 to form $X_1X_2X_3X_4X_5X_{93}X_{94}X_{95}X_{96}X_{97}$, wherein X_{93} , X_{94} and X_{97} may be any amino acid, X_{95} is selected from the group consisting of glutamine, glutamic acid, alanine and lysine, and X_{96} is a hydrophobic or aliphatic amino acid.
37. The amino acid sequence according to claim 36 wherein X_{93} is selected from the group consisting of alanine, aspartic acid, glutamic acid, arginine, and valine, X_{95} is glutamine or glutamic acid, and X_{96} is selected from the group consisting of leucine, isoleucine, valine and tryptophan.
38. The amino acid sequence according to claim 37 wherein X_{96} is leucine or tryptophan.
39. The amino acid sequence according to claim 38 wherein X_{96} is leucine.
40. The amino acid sequence according to claim 39 wherein X_{95} is glutamine, and X_{96} is tryptophan.
41. The amino acid sequence according to claim 40 wherein X_{93} is valine.
42. The amino acid sequence according to claim 41 wherein asparagine is bound to the amino terminal of X_1 .
43. An amino acid sequence selected from the amino acid sequences listed in Figures 1-A through 1-O.

44. The amino acid sequence according to claim 25 wherein the sequence is selected from the group consisting of FHENFYDWFVRQVS, DYKDVTFSTSAVFHENFYDWFVRQVSKK, GRVDWLQRNANFYDWFVAELG and APTFYAWFNQQT.
- 5 45. The amino acid sequence according to claim 25 wherein the sequence comprises FHENFYDWFVRQVS.
46. The amino acid sequence according to claim 25 wherein the sequence is selected from the group consisting of
10 FHENFYDWFVRQVAKK-NH₂
FHENFYDWFVRQASKK-NH₂
FHENFYDWFVRAVSKK-NH₂
FHENFYDWFVAQVSKK-NH₂
FHENFYDWFARQVSKK-NH₂
FHEAFYDWFVRQVSKK-NH₂
15 FHANFYDWFVRQVSKK-NH₂
FAENFYDWFVRQVSKK-NH₂
AHENFYDWFVRQVSKK-NH₂
fhenfydwfvrqvskk
EFHENFYDWFVRQVSEE
20 FHENFYGWFVRQVSKK
HETFYSMIRSLAK
SDGFYNAIELLS
SLNFYDALQLLAKK
HDPFYSMMKSLLK
25 NSFYEALRMLSSK
HPTSKEIYAKLLK
HPSTNQMLMKLKF
HPPLSELKFLIKK
HAPLSVLVQALLKK
30 HPSLSDMRWILLK
WSDFYSYFQGLD
D117-Dap(D117)
SSNFYQALMLLS
D117-Dap(CO-CH₂-O-NH₂)
35 HENFYGWFVRQVSKK
D117-Lys(D117)

D117-b-Ala-Lys(D117)
D117-b-Ala-Dap(b-Ala-D117)
D117-Gly-Lys(Gly-D117)
D117-b-Ala-Lys(b-Ala-D117)
5 D117-Dab(D117)
D117-Orn(D117)
D117-Dap(b-Ala-D117)
D117-b-Ala-Orn(b-Ala-D117)
10 L-(Thia-b-Ala-D117)₂
FHENFYDWFVRQVS
FHENFYDWFVRQVSK
FHENFYDWFVQVSK
FHENFYDWFVVS
15 FHENFYDWFVSK
FHENFYDWFVK
FYDWF-NH₂
FYDWFKK-NH₂
AFYDWFACK-NH₂
AAAAFYDWFAAAAKK-NH₂
20 (D117)₂₋₁₂
(Cys-Gly-D117)₂
Cys-Gly-D117
(D117)₂₋₁₄
LDALDRLMRIFYEERPSL-NH₂
25 PLAELWAYFEHSEQGRSSAH-NH₂
GRVDWLQRNANFYDWFVAELG-NH₂
NGVERAGTGDNFYDWFVAQLH-NH₂
EHWNTVDPFYFTLFEWLRESG-NH₂
EHWNTVDPFYQYFSELLRESG-NH₂
30 QSDSGTVHDRFYGWFRDTWAS-NH₂
AFYDWFACK-NH₂
AFYDWFANH₂
AFYDWF-NH₂
FYDWDANH₂
35 Ac-FYDWF-NH₂
Lig-FHENFYDWFVRQVSKK
Lig-GGGFHENFYDWFVRQVSKK
FHENFYDWFVRQVSKKGGG-Lig
Lig-CAWPTYWNCG
40 ACAWPTYWNCG-Lig
ACAWPTYWNCGGGG-Lig
Lig-SDGFYNAILLS
SDGFYNAILLS-Lig

SDGFYNAIELLSGGG-Lig
 KHLCVLEELFWGASLFGYCSGKK-Lig
 AFYDWFACK-Lig
 AFYEWFAKK-NH₂
 5 AFYGWFAKK-NH₂
 AFYKWFACK-NH₂
 (SDGFYNAIELLS-Lig)₂-14
 (AFYDWFACK-Lig)₂-14
 FHENAYDWFVRQVSKK
 10 FHENFADWFVRQVSKK
 FHENFYAWFVRQVSKK
 FHENFYDAFVRQVSKK
 FHENFTDWA VRQVSKK
 FQSLLEELVWGAPLFRYGTG
 15 PLCVLEELFWGASLFGQCSG
 QLEEEWAGVQCEVYGREPCS
 Cys-(Gly)₂-D117
 (Cys-(Gly)₂-D117),
 (S210)-14-(S212)
 20 (S131)-14-(S212)
 (S205)₂-14
 (S204)₂-14
 (S131)-14-(S210)
 RVDWLQRNANFYDWFVAELG
 25 VDWLQRNANFYDWFVAELG
 DWLQRNANFYDWFVAELG
 WLQRNANFYDWFVAELG
 LQRNANFYDWFVAELG
 QRNANFYDWFVAELG
 30 RNANFYDWFVAELG
 NANFYDWFVAELG
 ANFYDWFVAELG
 NFYDWFVAELG
 GRVDWLQRNANFYDWFVAELG-Lig
 35 Lig-GRVDWLQRNANFYDWFVAELG
 (S208)-14-(S131)
 (S208)-14-(S209)
 GRVDWLQRNANFYDWFVAEL
 GRVDWLQRNANFYDWFVAE
 40 GRVDWLQRNANFYDWFVA
 GRVDWLQRNANFYDWFV
 14-(SDGFYNAIELLS-Lig)₂
 (GRVDWLQRNANFYDWFVAELG)-14

- 14-(GRVDWLQRNANFYDWFVAE LG)
(SDGFYNAIELLSGGG)₂-14
H-Acy-CLEE-w-GASL-Tic-QCSG-NH₂
RWPNFYGYFESLLTHFS-NH₂
- 5 HYNIFYEYFQVLLAETW-NH₂
EGWDFYSYFSGLLASVT-NH₂
LDRQFYRYFQDLLVGFM-NH₂
WGRSFYRYFETLLAQGI-NH₂
PLCFLQELFGGASLGGYCSG-NH₂
- 10 WLEQERAWIWCEIQSGSCRA-NH₂
IQGWEPFYGWFDDEVVAQMFEENH₂
TGHRLGLDEQFYWWFRDALSG-NH₂
H-Abu-CLEE-w-GASL-Tic-QCSG-NH₂
14-(Dap-CAWPTYWNCG)₂
- 15 RDHypFYDWFDDi-NH₂
S131-14-S209
S294-14-S210
S295-14-S210
S294-14-204
- 20 S295-14-S204
GFREGQRWYWFVAQVT-NH₂
VASGHVLHGQFYRWFVDQFALEENH₂
VGDFCVSHDCFYGWFLRESMQ-NH₂
DLRVLCLEFGGAYVLGYCSE-NH₂
- 25 HLSVGEELSWWVALLGQWAR-NH₂
APVSTEELRWGALLFGQWAG-NH₂
ALEEEWAWVQVRSIRSGPL-NH₂
WLEHEWAIQCELYGRGCTY-NH₂
AAVHEQFYDWFADQYEE-NH₂
- 30 QAPSNFYDWFVREWDEENH₂
QSFYDYIEELGGEWKK-NH₂
DPFYQGLWEWLRESGEE-NH₂
(S204)₂-7
(S204)₂-9
- 35 (S204)₂-12
(S204)₂-13
DWLQRNANFYDWFVAEL-Lig
Lig-DWLQRNANFYDWFVAEL
(S209)₂-9
- 40 (S210)₂-9
LigKHL CVLEELFWGASLFGYCSGKKKK
KHL CVLEELFWGASLFGYCSGKKKK-Lig
(S294)₂-14

(S295)₂₋₁₄

S-D-G-F-Y-N-A-Acy-E-L-L-S

S-G-P-F-Y-E-E-Acy-E-L-L-W-Aib

G-G-S-F-Y-D-D-Acy-E-Aib-L-W-Aib

5 N-Aib-P-F-Y-D-E-Acy-D-E-Cha-W-Aib

GRVDWLQRNANFYDWFVAEAcyG-NH₂

and wherein underlined numbers represent a linker as defined in Table 18.

47. An amino acid sequence which specifically binds IR such that binding to IGF-1R is at or below background and wherein said amino acid sequence
10 comprises X₁X₂X₃X₄X₅ wherein X₁, X₂, and X₅ are selected from the group consisting of phenylalanine and tyrosine, X₃ is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X₄ is selected from group consisting of tryptophan, tyrosine and phenylalanine.
48. A method of modulating insulin activity in mammalian cells, said method comprising administering to said cells an amino acid sequence which binds
15 IR and comprises the sequence of amino acids X₆X₇X₈X₉X₁₀X₁₁X₁₂X₁₃ wherein X₆ and X₇ are aromatic amino acids or glutamine, X₈, X₉, X₁₁ and X₁₂ may be any amino acid, X₁₀ and X₁₃ are hydrophobic amino acids.
49. The method according to claim 48 wherein X₆ and X₇ are selected from
20 group consisting of phenylalanine and tyrosine, and X₁₀ and X₁₃ are selected from group consisting of leucine, isoleucine, tryptophan, phenylalanine methionine and valine.
50. The method according to claim 48 wherein X₆ is phenylalanine and X₇ is tyrosine.
- 25 51. The method according to claim 50 wherein X₁₀ is isoleucine.
52. The method according to claim 50 wherein X₁₀ is leucine.

53. The method according to claim 50 wherein X_{13} is leucine.
54. The method according to claim 50 wherein X_9 is tyrosine and X_{10} is phenylalanine.
55. The method according to claim 50 wherein the amino acid sequence is selected from $FYX_8X_9LX_{11}X_{12}L$, $FYX_8X_9IX_{11}X_{12}L$ and $FYX_8YFX_{11}X_{12}L$.
56. The method according to claim 55 wherein the amino acid sequence comprises $FYX_8X_9LX_{11}X_{12}L$.
57. The method according to claim 55 wherein the amino acid sequence comprises $FYX_8YFX_{11}X_{12}L$.
58. The method according to claim 48 wherein the amino acid sequence $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$ further comprises amino acids X_{98} and X_{99} at the amino terminal end and X_{100} at the carboxy terminal end to form $X_{98}X_{99}X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{100}$ and wherein X_{98} is optionally aspartic acid and X_{99} is independently an amino acid selected from the group consisting of glycine, glutamine and proline, and X_{100} is a hydrophobic amino acid.
59. The method according to claim 58 wherein X_{100} is an aliphatic amino acid.
60. The method according to claim 59 wherein X_{100} is leucine.
61. The method according to claim 48 wherein the amino acid sequence binds to the insulin receptor with an affinity of at least about 10^{-5} M.

62. The method according to claim 61 wherein the affinity is between about 10^{-7} M.
63. The method according to claim 48 wherein the amino acid sequence comprises DYKDFYDAIDQLVRGSARAGGTRD or
5 KDRAFYNGLRDLVGAVYGAWD.
64. The method according to claim 48 wherein the amino acid sequence is selected from the group of amino acid sequences listed in Figures 2A through 2P.
65. An amino acid sequence comprising $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$ wherein X_6 and X_7 are aromatic amino acids or glutamine, X_8 , X_9 , X_{11} and X_{12} may be
10 any amino acid, X_{10} and X_{13} are hydrophobic amino acids and wherein said amino acid sequence binds to IGF-1R.
66. The amino acid sequence according to claim 65 wherein the binding occurs at an affinity (K_d) of at least about 10^{-5} M.
67. The amino acid sequence according to claim 66 wherein the binding occurs
15 at an affinity (K_d) of at least about 10^{-7} M.
68. The amino acid sequence according to claim 65 wherein X_6 and X_7 are phenylalanine or tyrosine, and X_{10} and X_{13} are leucine, isoleucine, tryptophan, phenylalanine or methionine.
69. The amino acid sequence according to claim 68 wherein X_6 is phenylalanine
20 and X_7 is tyrosine.

70. The amino acid sequence according to claim 68 wherein X_{10} is isoleucine.
71. The amino acid sequence according to claim 68 wherein X_{10} is leucine.
72. The amino acid sequence according to claim 69 wherein X_{13} is leucine.
73. The amino acid sequence according to claim 69 wherein X_9 is tyrosine and
5 X_{10} is phenylalanine.
74. The amino acid sequence according to claim 68 wherein the amino acid
sequence comprises an amino acid sequence selected from
 $FYX_8X_9LX_{11}X_{12}L$, $FYX_8X_9IX_{11}X_{12}L$ and $FYX_8YFX_{11}X_{12}L$.
75. The amino acid sequence according to claim 74 wherein the amino acid
10 sequence comprises $FYX_8X_9IX_{11}X_{12}L$.
76. The amino acid sequence according to claim 74 wherein the amino acid
sequence comprises $FYX_8X_9LX_{11}X_{12}L$.
77. The amino acid sequence according to claim 74 wherein the amino acid
sequence is $FYX_8YFX_{11}X_{12}L$.
- 15 78. The amino acid sequence according to claim 65 wherein the amino acid
sequence $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$ further comprises amino acids X_{98} and
 X_{99} at the amino terminal end and X_{100} at the carboxy terminal end to form
 $X_{98}X_{99}X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{100}$ and wherein X_{98} is optionally aspartic
20 acid and X_{99} is independently an amino acid selected from the group
consisting of glycine, glutamine and proline, and X_{100} is a hydrophobic
amino acid.

79. The amino acid sequence according to claim 78 wherein X_{100} is an aliphatic amino acid.
80. The amino acid sequence according to claim 79 wherein X_{100} is leucine.
81. The amino acid sequence according to claim 68 wherein the amino acid sequence comprises DYKDFYDAIDQLVRGSARAGGTRD or
5 KDRAFYNGLRDLVGAVYGAWDKK.
82. The sequence according to claim 81 wherein the amino acid sequence comprises DYKDFYDAIDQLVRGSARAGGTRD.
83. An amino acid sequence comprising an amino acid sequence selected from the group consisting of amino sequences listed in Figures 2A through 2P.
10
84. An amino acid sequence comprising a sequence selected from the group consisting of
15 SFYEAHQLLGV,
NSFYEARMLSS,
SLNFDALQLLA,
SSNFYQALMLLS,
SDGFYNAIELLS,
HETFYSMIRSLA,
20 HDPFYSMKSL and
WSDFYSYFQGLD.

85. The amino acid sequence according to claim 65 wherein the sequence comprises the amino acid sequence
 $X_{115}X_{116}X_{117}X_{118}FYX_8YFX_{11}X_{12}LX_{119}X_{120}X_{121}X_{122}$ wherein X_{115} is selected from the group consisting of tryptophan, glycine, aspartic acid, glutamic acid and arginine, X_{116} is selected from the group consisting of aspartic acid, histidine, glycine and asparagine, X_{117} and X_{118} are selected from the group consisting of glycine, aspartic acid, glutamic acid, asparagine, and alanine, X_8 is selected from the group consisting of arginine, glycine, glutamic acid and serine, X_{11} is selected from the group consisting of glutamic acid, asparagine, glutamine and tryptophan, X_{12} is selected from the group consisting of aspartic acid, glutamic acid, glycine, lysine, and glutamine, X_{119} is selected from the group consisting of glutamic acid, glycine, glutamine, aspartic acid and alanine, X_{120} is selected from the group consisting of glutamic acid, aspartic acid, glycine and glutamine, X_{121} is selected from the group consisting of tryptophan, tyrosine, glutamic acid, phenylalanine, histidine and aspartic acid, and X_{122} is selected from the group consisting of glutamic acid, aspartic acid, and glycine.
86. The amino acid sequence according to claim 85 wherein X_{115} is tryptophan, X_{117} is selected from glycine, aspartic acid, glutamic acid and asparagine; X_{118} is selected from glycine, aspartic acid, glutamic acid and alanine; X_{119} , X_{120} , and X_{122} are glutamic acid; X_{12} is aspartic acid, and X_{121} is tryptophan or tyrosine.
87. An amino acid sequence comprising $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$ wherein X_6 and X_7 are aromatic amino acids or glutamine, X_8 , X_9 , X_{11} and X_{12} may be any amino acid, X_{10} and X_{13} are hydrophobic amino acids and wherein said amino acid sequence binds to IR such that binding to IGF-1R is at or below background.

88. A method of binding to Site 1 of IR from mammalian cells, said method comprising contacting IR with an amino acid sequence which binds IR and comprises the sequence of $X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}$ wherein X_{14} , X_{17} , and X_{18} are hydrophobic amino acids, X_{15} , X_{16} , and X_{19} are any amino acid, and X_{20} and X_{21} are aromatic amino acids.
89. The method according to claim 88 wherein X_{14} and X_{17} are selected from the group consisting of leucine, isoleucine and valine; X_{20} is selected from group consisting of tyrosine and histidine; and X_{21} is selected from group consisting of phenylalanine and tyrosine.
90. The method according to claim 89 wherein X_{14} and X_{17} are leucine.
91. The method according to claim 89 wherein X_{14} is leucine.
92. The method according to claim 89 wherein X_{17} is leucine.
93. The method according to claim 89 wherein X_{20} is tyrosine.
94. The method according to claim 89 wherein X_{21} is phenylalanine.
95. The method according to claim 90 wherein X_{15} is a large amino acid.
96. The method according to claim 89 wherein said amino acid sequence further comprises an amino acid extension comprising $X_{101}X_{102}X_{103}$ wherein X_{103} is bound to X_{14} at the amino terminus and X_{101} and X_{102} are polar amino acids and X_{103} is a hydrophobic amino acid.
97. The method according to claim 96 wherein X_{101} and X_{102} are independently aspartic acid or glutamic acid and X_{103} is leucine, isoleucine or valine.

98. A method of binding to Site 1 of IGF-1R from mammalian cells, said method comprising contacting IGF-1R with an amino acid sequence which binds IR and comprises the sequence of $X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}$ wherein X_{14} , X_{17} , and X_{18} are hydrophobic amino acids, X_{15} , X_{16} , and X_{19} are any amino acid, and X_{20} and X_{21} are aromatic amino acids.
99. The method according to claim 98 wherein X_{14} and X_{17} are selected from the group consisting of leucine, isoleucine and valine; X_{18} is an aromatic amino acid; X_{20} is selected from group consisting of tyrosine and histidine; and X_{21} is selected from group consisting of phenylalanine and tyrosine.
100. The method according to claim 98 wherein the amino acid sequence comprises a sequence selected from the sequences in Figures 3A through 3D.
101. An amino acid sequence which binds Site 1 of IR from mammalian cells, said sequence comprising $X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}$ wherein X_{14} , X_{17} , and X_{18} are hydrophobic amino acids, X_{15} , X_{16} , and X_{19} are any amino acid, and X_{20} and X_{21} are aromatic amino acids.
102. The amino acid sequence according to claim 101 wherein X_{14} and X_{17} are selected from the group consisting of leucine, isoleucine and valine; X_{20} is selected from group consisting of phenylalanine and tyrosine.
103. The amino acid sequence according to claim 102 wherein X_{14} and X_{17} are leucine.
104. The amino acid sequence according to claim 102 wherein X_{14} is leucine.
105. The amino acid sequence according to claim 102 wherein X_{17} is leucine.

106. The amino acid sequence according to claim 102 wherein amino acid X_{18} is tryptophan.
107. The amino acid sequence according to claim 103 wherein X_{20} is tyrosine.
108. The amino acid sequence according to claim 107 wherein X_{21} is phenylalanine.
- 5
109. The amino acid sequence according to claim 103 wherein X_{15} is a large amino acid.
110. The amino acid sequence according to claim 101 wherein at least one amino acid is a D-amino acid.
- 10
111. The amino acid sequence according to claim 65 wherein at least one amino acid is a D-amino acid.
112. The amino acid sequence according to claim 102 wherein said amino acid sequence further comprises an amino acid extension comprising $X_{101}X_{102}X_{103}$ wherein X_{103} is bound to X_{14} at the amino terminus and X_{101} and X_{102} are polar amino acids and X_{103} is a hydrophobic amino acid.
- 15
113. The amino acid sequence according to claim 112 wherein X_{101} and X_{102} are independently aspartic acid or glutamic acid and X_{103} is leucine, isoleucine or valine.

114. An amino acid sequence which binds Site 1 of IGF-1R from mammalian cells such that binding to IR is at or below background, said sequence comprising $X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}$ wherein X_{14} , X_{17} , and X_{18} are hydrophobic amino acids, X_{15} , X_{16} , and X_{19} are any amino acid, and X_{20} and X_{21} are aromatic amino acids.
115. The amino acid sequence according to claim 114 wherein X_{14} and X_{17} are selected from the group consisting of leucine, isoleucine and valine; X_{18} is an aromatic amino acid; X_{20} is selected from group consisting of tyrosine and histidine; and X_{21} is selected from group consisting of phenylalanine and tyrosine.
116. A method of binding to Site 2 of IR from mammalian cells, said method comprising contacting said cells with an amino acid sequence comprising $X_{22}X_{23}X_{24}X_{25}X_{26}X_{27}X_{28}X_{29}X_{30}X_{31}X_{32}X_{33}X_{34}X_{35}X_{36}X_{37}X_{38}X_{39}X_{40}X_{41}$ wherein X_{22} , X_{25} , X_{26} , X_{28} , X_{29} , X_{30} , X_{33} , X_{34} , X_{35} , X_{37} , X_{38} , X_{40} and X_{41} are any amino acid; X_{23} is any hydrophobic amino acid; X_{27} is a polar amino acid; X_{31} is an aromatic amino acid; X_{32} is a small amino acid; and wherein at least one cysteine is located at positions X_{24} through X_{27} and one at X_{39} or X_{40} .
117. The method according to claim 116 wherein X_{24} and X_{39} are cysteines.
118. The method according to claim 117 wherein X_{23} is selected from leucine, isoleucine, methionine and valine; X_{27} is selected from glutamic acid, aspartic acid, asparagine, and glutamine; X_{31} is tryptophan, X_{32} is glycine, and X_{36} is any aromatic amino acid.
119. The method according to claim 118 wherein the binding to IR occurs at an affinity (K_d) of at least about 10^{-5} M.

120. The method according to claim 116 wherein X_{23} is leucine, X_{27} is glutamic acid, X_{31} is tryptophan, and X_{32} is glycine.
121. The method according to claim 116 wherein the amino acid sequence is HLCVLEELFWGASLFGYCSG.
- 5 122. An amino acid sequence which binds IR, said amino acid sequence comprising
 $X_{22}X_{23}X_{24}X_{25}X_{26}X_{27}X_{28}X_{29}X_{30}X_{31}X_{32}X_{33}X_{34}X_{35}X_{36}X_{37}X_{38}X_{39}X_{40}X_{41}$
wherein X_{22} , X_{25} , X_{26} , X_{28} , X_{29} , X_{30} , X_{33} , X_{34} , X_{35} , X_{37} , X_{38} , X_{40} and X_{41} are
10 any amino acid, X_{23} is any hydrophobic amino acid, X_{27} is a polar amino acid; X_{31} is an aromatic amino acid; X_{32} is a small amino acid, and wherein at least one cysteine is located at positions X_{24} through X_{27} and one at X_{39} or X_{40} .
123. The amino acid sequence according to claim 122 wherein X_{24} and X_{39} are cysteines.
- 15 124. The amino acid sequence according to claim 123 wherein X_{23} is selected from methionine, valine, and leucine; X_{27} is selected from glutamic acid, alanine, glycine, glutamine, aspartic acid and valine; X_{31} and X_{32} are small amino acids; and X_{36} is an aromatic amino acid.
125. The amino acid sequence according to claim 122 wherein the binding to IR
20 occurs at an affinity (K_d) of at least about 10^{-5} M.
126. The amino acid sequence according to claim 124 wherein X_{23} is leucine, X_{27} is glutamic acid, X_{31} is tryptophan, and X_{32} is glycine.

127. The amino acid sequence according to claim 122 wherein the amino acid sequence is HLCVLEELFWGASLFGYCSG.
128. A method of modulating insulin activity in mammalian cells, said method comprising administering to said cells an amino acid sequence which binds IR and comprises the sequence $X_{42} X_{43} X_{44} X_{45} X_{46} X_{47} X_{48} X_{49} X_{50} X_{51} X_{52} X_{53} X_{54} X_{55} X_{56} X_{57} X_{58} X_{59} X_{60} X_{61}$ wherein X_{42} , X_{43} , X_{44} , X_{45} , X_{53} , X_{55} , X_{56} , X_{58} , X_{60} and X_{61} are any amino acid; X_{43} , X_{46} , X_{49} , X_{50} and X_{54} are hydrophobic amino acids; X_{47} and X_{59} are cysteines; X_{48} is a polar amino acid; X_{51} , X_{52} and X_{57} are small amino acids.
129. The method according to claim 128 wherein X_{43} and X_{46} are leucine; X_{48} is selected from the group consisting of aspartic acid and glutamic acid; X_{50} is phenylalanine or tyrosine; and X_{51} , X_{52} and X_{57} are glycine.
130. The method according to claim 129 wherein X_{48} is glutamic acid and X_{50} is a phenylalanine.
131. The method according to claim 130 wherein the amino acid sequence is $X_{42} X_{43} X_{44} X_{45} LCE X_{49} FGG X_{53} X_{54} X_{55} X_{56} GX_{58} C X_{60} X_{61}$.
132. The method according to the claim 131 wherein the amino acid sequence comprises DLRVLCELFGGAYVLGYCSE or DLRVLCELFGGAYVRGYCSE.
133. The method according to claim 128 wherein the binding to IR occurs at an affinity (K_d) of at least about 10^{-5} M.

134. An amino acid sequence which binds IR, said amino acid sequence comprising X₄₂ X₄₃ X₄₄ X₄₅ X₄₆ X₄₇ X₄₈ X₄₉ X₅₀ X₅₁ X₅₂ X₅₃ X₅₄ X₅₅ X₅₆ X₅₇ X₅₈ X₅₉ X₆₀ X₆₁ wherein X₄₂, X₄₃, X₄₄, X₄₅, X₅₃, X₅₅, X₆₀ and X₆₁ are any amino acid; X₄₃, X₄₆, X₄₉, X₅₀ and X₅₄ are hydrophobic amino acids; X₄₇ and X₅₉ are cysteines; X₄₈ is a polar amino acid; and X₅₁, X₅₂ and X₅₇ are small amino acids.
135. The amino acid sequence according to claim 134 wherein X₄₃ and X₄₆ are leucine; X₄₈ is selected from the group consisting of aspartic acid and glutamic acid; X₅₀ is phenylalanine or tyrosine; and X₅₁, X₅₂ and X₅₇ are glycine.
136. The amino acid sequence according to claim 135 wherein X₄₈ is glutamic acid and X₅₀ is phenylalanine.
137. The amino acid sequence according to claim 136 wherein the amino acid sequence comprises X₄₃ X₄₄ X₄₅ LCE X₄₉ FGG X₅₃ X₅₄ X₅₅ X₅₆ G X₅₈ C X₆₀ X₆₁.
138. The amino acid sequence according to claim 137 wherein an amino acid sequence comprises DLRVLCELFGGAYVLGYCSE or DLRVLCELFGGAYVRGYCSE
139. A method of modulating insulin activity in mammalian cells, said method comprising administering to said cells an amino acid sequence comprising X₆₂ X₆₃ X₆₄ X₆₅ X₆₆ X₆₇ X₆₈ X₆₉ X₇₀ X₇₁ X₇₂ X₇₃ X₇₄ X₇₅ X₇₆ X₇₇ X₇₈ X₇₉ X₈₀ X₈₁ wherein X₆₂, X₆₅, X₆₆ X₆₈, X₆₉, X₇₁, X₇₃, X₇₆, X₇₇, X₇₈, X₈₀ and X₈₁ are any amino acid; X₆₃, X₇₀, and X₇₄ are hydrophobic amino acids; X₆₄ is a polar amino acid; X₆₇ and X₇₅ are aromatic amino acids; and X₇₂ and X₇₉ are cysteines.

140. The method according to claim 139 wherein X_{63} is selected from the group consisting of leucine, isoleucine, methionine and valine; X_{70} and X_{74} are selected from group consisting of valine, isoleucine, leucine and methionine; X_{64} is selected from group consisting of aspartic acid and glutamic acid; X_{67} is tryptophan; and X_{75} is selected from group consisting of tyrosine and tryptophan.
141. The method according to claim 140 wherein X_{66} is glutamic acid.
142. The method according to claim 141 wherein X_{63} is leucine.
143. The method according to claim 140 wherein X_{74} is valine.
144. The method according to claim 141 wherein X_{64} is a glutamic acid.
145. The method according to claim 141 wherein X_{75} is a tyrosine.
146. The method accord to claim 140 wherein the amino acid sequence comprises WLDQEWAWVQCEVYGRGCPS.
147. An amino acid sequence which binds IR, said amino acid sequence comprising X_{62} X_{63} X_{64} X_{65} X_{66} X_{67} X_{68} X_{69} X_{70} X_{71} X_{72} X_{73} X_{74} X_{75} X_{76} X_{77} X_{78} X_{79} X_{80} X_{81} wherein X_{62} , X_{65} , X_{66} X_{68} , X_{69} , X_{71} , X_{73} , X_{76} , X_{77} , X_{78} , X_{80} and X_{81} are any amino acid; X_{63} , X_{70} , and X_{74} are hydrophobic amino acids; X_{64} is a polar amino acid; X_{67} and X_{75} are aromatic amino acids; and X_{72} and X_{79} are cysteines.

148. The amino acid sequence according to claim 147 wherein X_{63} is selected from the group consisting of leucine, isoleucine, methionine and valine; X_{70} and X_{74} are selected from group consisting of valine, isoleucine, leucine and methionine; X_{64} is selected from group consisting of aspartic acid and glutamic acid; X_{67} is tryptophan; and X_{75} is selected from group consisting of tyrosine and tryptophan.
149. The amino acid sequence according to claim 148 wherein X_{66} is glutamic acid.
150. The amino acid sequence according to claim 149 wherein X_{63} is leucine.
151. The amino acid sequence according to claim 148 wherein X_{74} is valine.
152. The amino acid sequence according to claim 149 wherein X_{64} is glutamic acid.
153. The amino acid sequence according to claim 148 wherein X_{75} is a tyrosine.
154. The amino acid sequence accord to claim 148 wherein the amino acid sequence comprises WLDQEAWVQCEVYGRGCPs.
155. The amino acid sequence according to claim 148 wherein the affinity (K_d) of binding to IR is at least 10^{-5} M.
156. The amino acid sequence according to claim 148 wherein the amino acid sequence comprises a sequence selected from the sequences of Figures 6A-6F.

157. A method of modulating insulin activity in mammalian cells, said method comprising administering to said cells an amino acid sequence which binds IR and comprises $HX_{82}X_{83}X_{84}X_{85}X_{86}X_{87}X_{88}X_{89}X_{90}X_{91}X_{92}$ herein X_{82} is proline or alanine; X_{83} is a small amino acid; X_{84} is selected from the group consisting of leucine, serine and threonine; X_{85} is a polar amino acid; X_{86} is any amino acid; X_{87} is an aliphatic amino acid; X_{88} , X_{89} , X_{90} is any amino acid; and X_{91} and X_{92} are aliphatic amino acids.
158. The method according to claim 157 wherein X_{82} is proline; X_{83} is selected from the group consisting of proline, serine and threonine; X_{84} is leucine; X_{85} is selected from the group consisting of glutamic acid, serine, lysine and asparagine; X_{86} is a polar amino acid; X_{87} is selected from the group consisting of leucine, methionine and isoleucine; and X_{91} and X_{92} are leucines.
159. The method according to claim 158 wherein X_{83} is proline.
160. The method according to claim 158 wherein X_{85} is serine.
161. The method according to claim 158 wherein X_{86} is selected from the group consisting of histidine, glutamic acid, aspartic acid and glutamine.
162. The method according to claim 158 wherein X_{87} is leucine.
163. The method according to claim 158 wherein X_{92} is phenylalanine.
164. The method according to claim 160 wherein the amino acid sequence is $HPPLSX_{86}LX_{88}X_{89}X_{90}LL$.

165. The method according to claim 158 wherein the amino acid sequence is selected from the group consisting of HPPLEHLKAFLL, HPPLSELKLFLL, HPSLSDMRWILL, HPTSKEIYAKLL, HPTSKEIYAKLL, HPSTNQMLMKLF and HAPLSVLQALL.
- 5 166. An amino acid sequence which binds IR, said amino acid sequence comprising $HX_{82}X_{83}X_{84}X_{85}X_{86}X_{87}X_{88}X_{89}X_{90}X_{91}X_{92}$ herein X_{82} is proline or alanine; X_{83} is a small amino acid; X_{84} is selected from the group consisting of leucine, serine and threonine; X_{85} is a polar amino acid; X_{86} is any amino acid; X_{87} is an aliphatic amino acid; X_{88} , X_{89} , X_{90} is any amino acid; and X_{91} and X_{92} are aliphatic amino acids.
- 10
167. The amino acid sequence according to claim 166 wherein X_{82} is proline; X_{83} is selected from the group consisting of proline, serine and threonine; X_{84} is leucine; X_{85} is selected from the group consisting of glutamic acid, serine, lysine and asparagine; X_{86} is a polar amino acid; X_{87} is selected from the group consisting of leucine, methionine and isoleucine; and X_{91} and X_{92} are leucines.
- 15
168. The amino acid sequence according to claim 167 wherein X_{83} is proline.
169. The amino acid sequence according to claim 167 wherein X_{85} is serine.
170. The amino acid sequence according to claim 167 wherein X_{86} is selected from the group consisting of histidine, glutamic acid, aspartic acid and glutamine.
- 20
171. The amino acid sequence according to claim 167 wherein X_{87} is leucine.

172. The amino acid sequence according to claim 167 wherein X₉₂ is phenylalanine.
173. The amino acid sequence according to claim 169 wherein the amino acid sequence is HPPLSX₈₆ LX₈₈ X₈₉ X₉₀ LL.
- 5 174. The amino acid sequence according to claim 167 wherein the amino acid sequence is selected from the group consisting of HPPLEHLKAFLI, HPPLSELKLFLI, HPSLSDMRWILL, HPTSKEIYAKLL, HPTSKEIYAKLL, HPSTNQMLMKLF and HAPLSVLQALL.
- 10 175. A method modulating insulin activity in mammalian cells, said method comprising administering to said cells an amino acid sequence comprising an amino acid sequence of X₁₀₄X₁₀₅X₁₀₆X₁₀₇X₁₀₈X₁₀₉X₁₁₀X₁₁₁X₁₁₂X₁₁₃X₁₁₄ wherein at least one of the amino acids of X₁₀₆ through X₁₁₁ are tryptophan; wherein X₁₀₄ and X₁₁₄ are both small amino acids; wherein X₁₀₅ is any amino acid; and wherein at least one of X₁₀₄, X₁₀₅, X₁₀₆ and one of X₁₁₂ X₁₁₃ 15 X₁₁₄ are cysteine residues.
176. The method according to claim 175 wherein at least two of the amino acids of X₁₀₆ through X₁₁₁ are tryptophan which are separated from each other by at least two amino acids.
- 20 177. The method according to claim 176 wherein the separating amino acids are selected from the group consisting of proline, threonine and tyrosine.
178. The method according to claim 177 wherein the amino acid sequence comprises WPTYW.

179. The method according to claim 178 wherein X_{105} and X_{113} are cysteine residues.
180. The method according to claim 178 wherein X_{104} and X_{114} are selected from the group consisting of alanine and glycine.
- 5 181. The method according to claim 180 wherein X_{104} is alanine and X_{114} is glycine.
182. The method according to claim 181 wherein X_{105} is valine.
183. The method according to claim 182 wherein X_{112} is asparagine.
184. The method according to claim 198 wherein the affinity (K_d) of binding to
10 IR is at least about 10^{-5} M.
185. A method of modulating insulin activity in mammalian cells, said method comprising administering to said cells an amino acid sequence comprising an amino acid sequence selected from the group listed in Figure 8.
186. The method according to claim 185 wherein the sequence comprises
15 ACVWPTYWNCG.
187. An amino acid sequence which binds to IR and comprising an amino acid sequence of $X_{104}X_{105}X_{106}X_{107}X_{108}X_{109}X_{110}X_{111}X_{112}X_{113}X_{114}$ wherein at least one of the amino acids of X_{106} through X_{111} are tryptophan; wherein X_{104} and X_{114} are both small amino acids; wherein X_{105} is any amino acid; and
20 wherein at least one of X_{104} , X_{105} , X_{106} and one of X_{112} , X_{113} , X_{114} are cysteine residues.

188. The amino acid sequence according to claim 187 wherein at least two of the amino acids of X₁₀₆ through X₁₁₁ are tryptophan which are separated from each other by at least two amino acids.
189. The amino acid sequence according to claim 188 wherein the separating amino acids are selected from the group consisting of proline, threonine and tyrosine.
190. The amino acid sequence according to claim 189 wherein the amino acid sequence comprises WPTYW.
191. The amino acid sequence according to claim 190 wherein X₁₀₅ and X₁₁₃ are cysteine residues.
192. The amino acid sequence according to claim 190 wherein X₁₀₄ and X₁₁₄ are selected from the group consisting of alanine and glycine.
193. The amino acid sequence according to claim 190 wherein X₁₀₄ is alanine and X₁₁₄ is glycine.
194. The amino acid sequence according to claim 193 wherein X₁₀₅ is valine.
195. The amino acid sequence according to claim 194 wherein X₁₁₂ is asparagine.
196. The amino acid sequence according to claim 202 wherein the affinity (K_d) of binding to IR is at least about 10⁻⁵ M.
197. An amino acid sequence which binds IR from mammalian cells comprising an amino acid sequence selected from the group listed in Figure 8.

198. The amino acid sequence according to claim 197 comprising
ACVWPTYWNCG.
199. A method of providing insulin agonist activity to mammalian cells, said
method comprising administering to said cells an amino acid sequence
comprising DYKDLCSWGVRIGWLAGLCPKK.
200. A method of modulating insulin activity in mammalian cells, said method
comprising administering to said cells an amino acid sequence comprising
an amino acid sequence selected from the group listed in Figures 9 through
11.
201. An amino acid sequence comprising DYKDLCSWGVRIGWLAGLCPKK.
202. An amino acid sequence comprising an amino acid sequence selected from
the group listed in Figures 9 through 11.
203. An amino acid sequence comprising at least two amino acid sequences
which independently bind IR, with the proviso that at least one of the
sequences is not insulin or a fragment thereof.
204. The amino acid sequence according to claim 203 wherein the two amino
acid sequences bind to Site 1 of IR.
205. The amino acid sequence according to claim 203 wherein one amino acid
sequence binds to Site 1, and the other binds to Site 2 of IR.

206. The amino acid sequence according to claim 203, wherein at least one of the sequences is selected from the group consisting of $X_1X_2X_3X_4X_5$ wherein X_1 , X_2 , X_4 , and X_5 are aromatic amino acids, and X_3 may be any polar amino acid; $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$ wherein X_6 and X_7 are aromatic amino acids or glutamine, X_8 , X_9 , X_{11} and X_{12} may be any amino acid, X_{10} and X_{13} are hydrophobic amino acids; and $X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}$ wherein X_{14} , X_{17} , and X_{18} are hydrophobic amino acids, X_{15} , X_{16} , and X_{19} are any amino acid, and X_{20} and X_{21} are aromatic amino acids.
207. The amino acid sequence according to claim 206, wherein at least one of the sequences is $X_1X_2X_3X_4X_5$ wherein X_1 , X_2 , X_4 , and X_5 are aromatic amino acids, and X_3 may be any polar amino acid.
208. The amino acid sequence according to claim 206 wherein at least one of the sequences comprises FYX_3WF .
209. The amino acid sequence according to claim 206, wherein at least one of the sequences comprises $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$ wherein X_6 and X_7 are aromatic amino acids or glutamine, X_8 , X_9 , X_{11} and X_{12} may be any amino acid, X_{10} and X_{13} are hydrophobic amino acids.
210. The amino acid sequence according to claim 209, wherein at least one of the sequences comprises $FYX_8X_9LX_{11}X_{12}L$.
211. The amino acid sequence according to claim 206, wherein at least one of the sequences comprises $X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}$ wherein X_{14} , X_{17} , and X_{18} are hydrophobic amino acids, X_{15} , X_{16} , and X_{19} are any amino acid, and X_{20} and X_{21} are aromatic amino acids.

212. The amino acid sequence according to claim 211 wherein at least one of the sequences comprises LX₁₅, X₁₆, LLX₁₉YF.

213. The amino acid sequence according to claim 203 wherein at least one of the sequences comprises

5 X₂₂X₂₃X₂₄X₂₅X₂₆X₂₇X₂₈X₂₉X₃₀X₃₁X₃₂X₃₃X₃₄X₃₅X₃₆X₃₇X₃₈X₃₉X₄₀X₄₁
 wherein X₂₂, X₂₅, X₂₆, X₂₈, X₂₉, X₃₀, X₃₃, X₃₄, X₃₅, X₃₆, X₃₇, X₃₈, X₄₀, and X₄₁ are any amino acid, X₂₃ is any hydrophobic amino acid; X₂₇ is a polar amino acid; X₃₁ is an aromatic amino acid; X₃₂ is a small amino acid, and wherein at least one cysteine is located at positions X₂₄ through X₂₇ and one
 10 at X₃₉ or X₄₀; X₄₂ X₄₃ X₄₄ X₄₅ X₄₆ X₄₇ X₄₈ X₄₉ X₅₀ X₅₁ X₅₂ X₅₃ X₅₄ X₅₅
 X₅₆X₅₇X₅₈X₅₉ X₆₀ X₆₁ wherein X₄₂, X₄₃, X₄₄, X₄₅, X₅₃, X₅₅, X₅₆, X₅₈, X₆₀ and X₆₁ are any amino acid; X₄₃, X₄₆, X₄₉, X₅₀ and X₅₄ are hydrophobic amino acids; X₄₇ and X₅₉ are cysteine; X₄₈ is a polar amino acid; and X₅₁, X₅₂ and X₅₇ are small amino acids; or X₆₂ X₆₃ X₆₄ X₆₅ X₆₆ X₆₇ X₆₈ X₆₉ X₇₀ X₇₁ X₇₂
 15 X₇₃ X₇₄ X₇₅ X₇₆ X₇₇ X₇₈ X₇₉ X₈₀ X₈₁ wherein X₆₂, X₆₅, X₆₆ X₆₈, X₆₉, X₇₁, X₇₃, X₇₆, X₇₇, X₇₈, X₈₀ and X₈₁ are any amino acid; X₆₃, X₇₀, and X₇₄ are hydrophobic amino acids; X₆₄ is a polar amino acid; X₆₇ and X₇₅ are aromatic amino acids; and X₇₂ and X₇₉ are cysteines.

214. The amino acid sequence according to claim 203 wherein at least one of the sequences comprises HX₈₂X₈₃X₈₄X₈₅X₈₆X₈₇X₈₈X₈₉X₉₀X₉₁X₉₂ herein X₈₂ is proline or alanine; X₈₃ is a small amino acid; X₈₄ is selected from the group consisting of leucine, serine and threonine; X₈₅ is a polar amino acid; X₈₆ is any amino acid; X₈₇ is an aliphatic amino acid; X₈₈, X₈₉, X₉₀ is any amino acid; and X₉₁ and X₉₂ are aliphatic amino acids or

25 X₁₀₄X₁₀₅X₁₀₆X₁₀₇X₁₀₈X₁₀₉X₁₁₀X₁₁₁X₁₁₂X₁₁₃X₁₁₄ wherein at least one of the amino acids of X₁₀₆ through X₁₁₁ are tryptophan; wherein X₁₀₄ and X₁₁₄ are both small amino acids; wherein X₁₀₅ is any amino acid; and wherein at least one of X₁₀₄, X₁₀₅, X₁₀₆ and one of X₁₁₂ X₁₁₃ X₁₁₄ are cysteine residues.

215. The amino acid sequence according to claim 203 wherein the two amino acid sequences are connected by a peptide or non-peptide linker.

216. The amino acid sequence according to claim 215 wherein the linker is a peptide consisting of about 2 to about 16 amino acids.

5 217. The amino acid sequence according to claim 215 wherein the linker is a non-peptide.

218. The amino acid sequence according to claim 217 wherein the linker is dialdehyde.

10 219. The amino acid sequence according to claim 203 wherein the amino acid sequence is selected from the group consisting of

DYKDDDDKFHENFYDWFVRQVSGSGGLDALDRLMRYGEERPSLA
AAGAP,

DYKDDDDKFHENFYDWFVRQVSGGSHLCVLEELFWGASLFGYCSG
AAAGAPVPYPDPLEPRAA,

15 DYKDDDDKFHENFYDWFVRQVSGSGGGSGGSHLCVLEELFWGASL
FGYCSGAAAGAPVPYPDPLEPRAA,

DYKDDDDKFHENFYDWFVRQVSGSGGGSGGSHLCVLEELFWG
ASLFGYCSGAAAGAPVPYPDPLEPRAA,

20 AQPAMAFHENFYDWFVRQVSGGSFHENFYDWFVRQVSAAAGAPVP
YDPLEPRAA,

AQPAMAFHENFYDWFVRQVSGGSFHENFYDWFVRQVSGGSFHENF
YDWFVRQVSAAAGAPVPYPDPLEPRAA,

AQPAMAFHENFYDWFVRQVSGSGSGSFHENFYDWFVRQVSAAAG
APVPYPDPLEPRAA,

- 5 AQPAMAFHENFYDWFVRQVSGSGSGSGGSFHENFYDWFVRQVSAA
AGAPVPYPDPLEPRAA and

AQPAMAFHENFYDWFVRQVSGSGSGSGSGGSFHENFYDWFVRQV
SAAAGAPVPYPDPLEPRAA.

- 10 220. A nucleic acid sequence encoding amino acid sequence which binds to IR at
Site 1 and/or Site 2, with the proviso that the sequence is not insulin, IGF, or
fragments thereof.

- 15 221. The nucleic acid sequence according to claim 220 wherein the nucleic acid
sequence encodes for an amino acid sequence selected from the group
consisting of FYDWF, FYEWF, FHENFYDWF, FHENFYDWFVRQVSK,
DYKDVTFSTSAVFHENFYDWFVRQVSKK, GRVDWLQRNANFYDWFV
AELG and APTFYAWFNQQT.

- 20 222. The nucleic acid sequence according to claim 220 wherein the nucleic acid
sequence encodes for an amino acid sequence selected from the group
consisting of DYKDFYDAIDQLVRGSARAGGTRDKK and
KDRAFYNGLRDLVGAVYGAWDKK.

223. The nucleic acid sequence according to claim 220 wherein the nucleic acid
sequence encodes for an amino acid sequence selected from the group
consisting of SFYEAHQLLGV,

5 NSFYEALRMLSS,
 SLNFDALQLLA,
 SSNFYQALMLLS,
 SDGFYNAIELLS,
 HETFYSMIRSLA,
 HDPFYSMMKSL and
 WSDFYSYFQGL.

- 10 224. A kit for identifying a compound which binds IGF-1 receptor, comprising a
 IGF-1 receptor and an amino acid sequence selected from Formulas 1-10, or
 the amino acid sequences of Figures 9-11, which bind to the receptor at Site
 1 or Site 2.
225. The kit according to claim 224, wherein the amino acid sequence comprises
 the amino acid sequence FYDWF.
- 15 226. The kit according to claim 225, wherein the amino acid sequence comprises
 the amino acid sequence SAKNFYDWFVKK.
227. The kit according to claim 226 wherein the amino acid sequence comprises
 the amino acid sequence FYSLLASL.
228. The kit according to claim 227 wherein the amino acid sequence comprises
 the amino acid sequence QMKDIFYSLLASLAAKK.
- 20 229. A kit for identifying a compound which binds IR comprising IR and an
 amino acid sequence selected from Formulas 1-10 or the amino acid
 sequences of Figures 9 and 11 which bind IR at Site 1 or Site 2.
230. A pharmaceutical composition comprising a amino acid sequence which
 binds specifically to IGF-1 receptor at Site 1 and is an IGF agonist, with the
25 proviso that the amino acid sequence is not IGF-1, insulin, or fragments
 thereof, and a pharmaceutically acceptable carrier.

231. The composition according to claim 230, wherein the peptide comprises the amino acid sequence NFYDWFV.
232. The pharmaceutical composition according to claim 230, wherein the peptide comprises the amino acid sequence QMKDIFYSLLASLAA.
- 5 233. A pharmaceutical composition comprising a amino acid sequence which binds specifically to IR receptor at Site 1 and is an insulin agonist, with the proviso that the amino acid sequence is not insulin, IGF, or fragments thereof, and a pharmaceutically acceptable carrier.
- 10 234. The pharmaceutical composition according to claim 233, wherein the peptide comprises the amino acid sequence FYDWF.
235. The pharmaceutical composition according to claim 233, wherein the peptide comprises the amino acid sequence FYSLLASL.
- 15 236. A method of treating diabetes comprising administering to an individual in need of treatment a therapeutically effective amount of an amino acid sequence which binds IR at Site 1 and is an insulin agonist, with the proviso that the amino acid sequence is not insulin, IGF, or fragments thereof.
237. The method according to claim 236 wherein the amino acid sequence is expressed by a recombinant vector administered to the individual.
- 20 238. The method according to claim 236 wherein the amino acid sequence is administered to the individual as a polypeptide.

239. A method of treating a patient with an IGF sensitive tumor comprising administering to an individual in need of treatment a therapeutically effective amount of an amino acid sequence which is an IGF-1R antagonist, with the proviso that the amino acid sequence is not insulin, IGF, or fragments thereof.
240. The method according to claim 239 wherein the amino acid sequence is expressed by a recombinant vector administered to the individual.
241. The method according to claim 239 wherein the amino acid sequence is administered to the individual as a polypeptide.
242. A method of screening for a compound which binds to IR comprising:
- immobilizing IR, or a fragment thereof, on a surface;
 - incubating the IR, or fragment thereof, with a known amount of labeled amino acid sequence of Formulas 1-10, or an amino acid sequence selected from Figures 10-11, which binds IR and a compound to be screened under conditions which provide for binding of the labeled amino acid sequence to bind IR;
 - measuring the amount of labeled amino acid sequence bound to IR;
 - determining from the amount of bound labeled peptide whether the compound has competitively bound to IR.
243. An amino acid sequence capable of binding to Site 1 or Site 2 of IR identified by the method according to claim 242, with the proviso that the amino acid sequence is not insulin, IGF, or fragments thereof.
244. The amino acid sequence according to claim 243 wherein the amino acid sequence is an IR agonist.

245. The amino acid sequence according to claim 243 wherein the amino sequence binds to Site 1 of IR.
246. The amino acid sequence according to claim 243 wherein the amino sequence binds to Site 2 of IR.
- 5 247. A method of screening for a compound which binds to IGF-1R comprising:
- i) immobilizing IGF-1R, or a fragment thereof, on a surface;
 - ii) incubating the IGF-1R, or fragment thereof, with a known amount of labeled amino acid sequence of Formulas 1-9, or an amino acid sequence selected from Figure 10, which binds IGF-1R and a compound to be screened under
 - 10 conditions which provide for binding of the labeled amino acid sequence to bind to IGF-1R;
 - iii) measuring the amount of labeled amino acid sequence bound to IGF-1R;
 - iv) determining from the amount of bound labeled peptide
 - 15 whether the compound has competitively bound to IGF-1R.
248. An amino acid sequence capable of bind to Site 1 or Site 2 of IGF-1R identified by the method according to claim 247, with the proviso that the amino acid sequence is not insulin, IGF, or fragments thereof.
249. The amino acid sequence according to claim 248 wherein the amino acid sequence is an IGF agonist.
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250. The amino acid sequence according to claim 248 wherein the amino sequence binds to Site 1 of IGF-1R.

251. The amino acid sequence according to claim 248 wherein the amino sequence binds to Site 2 of IGF-1R.
252. An amino acid sequence comprising the sequence $WX_{123}GYX_{124}WX_{125}X_{126}$ wherein X_{123} is proline, glycine, serine, arginine, alanine or leucine, X_{124} is any amino acid; X_{125} is a hydrophobic amino acid; and X_{126} is any amino acid.
253. The amino acid sequence according to claim 252 wherein X_{123} is proline and X_{125} is leucine or phenylalanine.
254. A recombinant peptide library comprising members wherein the majority of the members comprise an amino acid sequence of Formula 1.
255. A recombinant peptide library comprising members wherein the majority of the members comprise an amino acid sequence of Formula 2.
256. A recombinant peptide library comprising members wherein the majority of the members comprise an amino acid sequence of Formula 3.
257. A recombinant peptide library comprising members wherein the majority of the members comprise an amino acid sequence of Formula 4.
258. A recombinant peptide library comprising members wherein the majority of the members comprise an amino acid sequence of Formula 5.
259. A recombinant peptide library comprising members wherein the majority of the members comprise an amino acid sequence of Formula 6.

260. A recombinant peptide library comprising members wherein the majority of the members comprise an amino acid sequence of Formula 7.
261. A recombinant peptide library comprising members wherein the majority of the members comprise an amino acid sequence of Formula 8.
- 5 262. A recombinant peptide library comprising members wherein the majority of the members comprise an amino acid sequence of Formula 9.
263. A recombinant peptide library comprising members wherein the majority of the members comprise an amino acid sequence of Formula 10.

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ABSTRACT OF THE DISCLOSURE

Peptide sequences capable of binding to insulin and/or insulin-like growth
5 factor receptors with either agonist or antagonist activity and identified from various
peptide libraries are disclosed. This invention also identifies at least two different
binding sites which are present on insulin and insulin-like growth factor receptors
which selectively bind the peptides of this invention. As agonists, certain of the
peptides of this invention may be useful for development as therapeutics to
10 supplement or replace endogenous peptide hormones. The antagonists may also be
developed as therapeutics.

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Clone Design	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGfsR	IR	IGFR/IR	IR/IGFR
R200-3-20D3-IR	XXXXXXXXXXXXXXXXXX	46.3	36.2	7.0	5.2	0.2
R200-3-20F1-IR	IGGQGHQDGNFYDMFEVALA	49.0	26.0	2.8	9.3	0.1
R200-3-20H1-IR	VFNCRSQDLDFENFEQAA	45.6	35.3	3.3	10.7	0.1
R200-3-20D1-IR	RVAGAIAPGLVKNQDGLFYSWFRE	50.8	37.5	3.0	12.5	0.1
R200-4-B12-IR	VLQARRHGCDVSDFYEWFA	41.9	2.9	5.7	0.5	2.0
R200-4-H3-IR	GAFYRWFEALVGSERVPDV	13.9	5.8	2.4	2.4	0.4
R200-4-D10-2-IR	HEAFYDMFSALVGGYELMG	21.5	7.3	2.9	2.5	0.4
R200-4-C6-IR	RIGGWARSEGIFYEFVREL	44.9	31.1	9.6	3.2	0.3
R200-4-E7-IR	LPAGGA?GFA?RGFYENFES	45.0	18.8	5.9	3.2	0.3
R200-4-E7-2-IR	GHSWALVRHVDRLFYEWFDL	45.0	18.8	5.9	3.2	0.3
R200-4-G3-IR	LGTSAQGVGHRAFYQWFQS	38.6	7.5	2.0	3.8	0.3
R200-4-H6-IR	RGGTIFYEFWESALRHKGAG	14.8	7.6	2.0	3.8	0.3
R200-4-G11-IR	NSGQGVVGLTFYSWFAQV	39.4	7.5	1.9	3.9	0.3
R200-4-G8-IR	FYGMFSRQLSLTRDDWGLP	41.2	15.1	3.4	4.4	0.2
R200-4-H9-IR	RMFYEFWFSQMGAPTEGSA	43.1	8.8	2.0	4.4	0.2
R200-4-H8-IR	IGGQGHQDGNFYDMFEVALA	47.9	43.7	9.3	4.7	0.2
R200-4-B8-IR	RDKEPDEEQNWSFYENFRH	44.0	40.1	8.4	4.8	0.2
R200-4-E2-IR	WSALLSWMDTGFYAFDDAV	16.3	13.9	2.4	5.8	0.2
R200-4-F4-IR	SRDQTNFTNSAGFYCWFER	15.3	5.9	1.0	5.9	0.2
R200-4-A8-IR	GVGLTMSDDAFYTFV	43.3	36.0	6.0	6.0	0.2
R200-4-C4-IR	IGGSFYEFYGFNDQV	17.3	26.8	4.3	6.2	0.2
R200-4-D7-IR	DIGSDGHRWDVSFYWFEM	44.8	36.2	5.6	6.5	0.2
R200-4-D2-IR	VLQARRHGCDVSDFYEWFA	31.2	29.4	2.9	10.1	0.1
	DPERMQSDGVGYEFWFEAVG					

Figure 1C

Clone Design	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGF5R	IR	IGF5R/IR	IR/IGF5R	--
A6S-3-E12-IR	XXXXXXXXXXNFYDFYXXXX	26.2	1.3	8.0	0.2	6.2	--
A6S-2-C1-IR	GRVDMLQRNANFYDMFVABLG	41.2	1.3	7.0	0.2	5.4	--
A6S-1-A7-IR	RVYFTGAPQNFYDMFVQEWED	47.2	2.3	11.1	0.2	4.8	--
A6S-2-C8-IR	HHQTGLQVQRNFYDMFVNEUR	44.9	1.5	5.5	0.3	3.7	--
A6S-3-E10-IR	MHRMOHDTGSNFYDMFVLOWA	46.9	1.6	5.0	0.3	3.1	--
A6S-2-U5-IR	AMHVVAQGGPNFYDMFVRELR	31.9	1.2	3.7	0.3	3.1	--
A6S-1-B2-IR	ATOMNGNLAFNFYDMFVRELT	31.6	1.8	5.3	0.3	2.9	--
A6S-1-A4-IR	TURKSVQEPNRYDMFVWAAAR	43.3	3.6	9.2	0.4	2.6	--
A6S-4-G3-IR	PHGHRGFAQNFYDMFVTOBE	31.3	2.3	5.1	0.5	2.2	--
A6S-4-H8-IR	RLASASVPGQNFYDMFVDOLL	11.5	1.7	3.6	0.5	2.1	--
A6S-3-E11-IR	RQSEPTLSNFYDMFVRELE	26.3	2.3	4.4	0.5	1.9	--
A6S-1-A1-IR	GOQLSTRVNFYDMFVQQLV	36.9	3.7	6.5	0.6	1.8	--
A6S-2-C9-IR	MSEPAVGVGNFYDMFVQAQLF	43.6	1.3	2.3	0.6	1.8	--
A6S-2-C4-IR	VGTRGLRLDNFYDMFVGVQYS	34.5	5.6	9.6	0.6	1.7	--
A6S-4-H10-IR	SREAVOKRNNANFYDMFVQOLS	39.2	4.4	6.9	0.6	1.6	--
A6S-4-G7-IR	LAQFAGSRNQNFYDMFVEQLG	19.1	1.4	2.2	0.6	1.6	--
A6S-4-H2-IR	GOEYFDQGLNFYDMFVRELD	25.5	2.6	3.9	0.7	1.5	--
A6S-2-C3-IR	RQSQPPHGSNFYDMFVEATN	31.1	1.6	2.4	0.7	1.5	--
A6S-2-C11-IR	LMQSLGSGSTNFYDMFVQQWV	20.9	3.3	4.6	0.7	1.4	--
A6S-3-F3-IR	DOORSACDGTNFYDMFVCOOLS	37.1	3.0	4.2	0.7	1.4	--
A6S-3-E5-IR	LOGTKACQRNFYDMFVQOTE	31.6	2.5	3.5	0.7	1.4	--
A6S-1-B7-IR	PEARTRVHHSNFYDMFVQAOLS	49.2	1.6	2.3	0.7	1.4	--
A6S-3-E7-IR	PWMLSVGIQDNFYDMFVGLDS	37.2	5.0	6.3	0.8	1.3	--
A6S-4-G6-IR	ASHQRGSSDNFYDMFVQAQMR	16.8	3.1	4.0	0.8	1.3	--
A6S-2-C2-IR	TLEREGEFSQNFYDMFVEQLH	29.7	2.4	3.1	0.8	1.3	--
A6S-3-F1-IR	DQSGTSGVHGDFYDMFVSALG	22.7	2.3	3.0	0.8	1.3	--
A6S-2-C5-IR	DMDKLGSLSNFYDMFVDQLA	42.9	6.1	7.0	0.9	1.1	--
A6S-3-B4-IR	VRVVLNQSGRNFYDMFVIOLE	20.9	2.1	2.3	0.9	1.1	--
A6S-3-B4-IR	MASMQSRTPDNFYDMFVRELS						--

Figure 1E

Clone	Design	Sequence	Ratios over Background		Comparisons	
			E-Tag	IR	IGF/IR	IR/IGF
		XXXXXXXXXXNFYDNFVXXXX	--	--	--	--
		TTCPRGDCNFYDNFVQQLR	36.6	9.0	8.9	1.0
		VRGDSVLRANFYDNFVQLR	36.7	6.8	6.9	1.0
		TPRSQVSRDHFNFYDNFVQLA	46.3	6.1	5.8	1.1
		ESLTGSRPDRNFYDNFVQOTS	37.0	5.3	5.1	1.0
		POSUTEVRKGNFYDNFVQWLH	42.7	5.2	5.1	1.0
		DVGMGRVKETNFYDNFVRLI	39.7	2.1	2.1	1.0
		GADDIRSLTNFYDNFVQVLS	18.6	3.1	2.9	1.1
		GVSIQAGYKTNFYDNFVEAVR	46.2	2.3	2.1	1.1
		VGEHRQMSVGNFYDNFVQJIA	31.2	2.0	1.7	1.2
		GSSLGRSGFGNFYDNFVQDLE	39.0	5.9	4.5	1.3
		HRQDVRQGNFYDNFVQVLE	44.8	4.3	3.3	1.3
		QUTFLTAREGNFYDNFVIRALE	33.5	3.6	2.7	1.3
		EAIWREEGQNFYDNFVQVLE	11.1	2.5	1.9	1.3
		VCDYSTGGTNFYDNFVQVVG	22.4	2.4	1.9	1.3
		PQPRASSTPLNFYDNFVQVATG	41.3	2.1	1.7	1.2
		GVSRGSGDPNFYDNFVQMLR	37.0	13.5	9.9	1.4
		GFGRHDSRGNFYDNFVEQLA	36.2	11.8	7.8	1.5
		ERFALEVQSGNFYDNFVQVLI	48.1	7.2	4.8	1.5
		NLKSSATVGGNFYDNFVEQL	18.3	3.6	2.6	1.4
		MEGPPAGGFLNFYDNFVQAVD	18.7	2.9	1.9	1.5
		RLDVAGHRGGNFYDNFVQKLIH	33.8	2.0	1.4	1.4
		PMSDHEALNQNFYDNFVSQVL	46.7	19.2	12.1	1.6
		EDRLNGESTNFYDNFVQVLA	36.9	18.2	10.7	1.7
		GKLVASTLDDNFYDNFVQVLS	32.8	12.8	7.9	1.6
		SGPVVQVTQGNFYDNFVQHLR	33.2	12.0	7.1	1.7
		VDRAGGAPDNFYDNFVQVOLD	33.9	10.8	6.8	1.6
		SILGRNDPDENFYDNFVSQVQ	44.3	9.6	5.7	1.7
		RVMATANAFNFYDNFVQVQLQ	23.2	4.3	2.5	1.7

Figure 1E (Con't)

Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGfsR IR	IGfsR/IR	IR/IGfsR
A6S-4-G1-IR	XXXXXXXXXXNFYDFVXXXX	--	--	--	--
A6S-1-A3-IR	NGVERAGTGDNFYDFVQAQLH	36.2	31.8	15.7	2.0
A6S-3-F12-IR	PFAGKGDKTGNFYDFVSLTG	39.9	12.6	6.0	2.1
A6S-4-G2-IR	GMPQYMDQVNFYDFVQAQVD	41.4	7.4	4.0	1.9
A6S-1-B1-IR	MGTPAVGDGANNFYDFVVRQLG	26.7	7.0	3.5	2.0
A6S-2-D11-IR	SKCKAWYGANNFYDFVWQVD	30.6	3.7	1.9	1.9
A6S-2-D1-IR	EAASLGSQDRNFYDFVVRQV	48.4	37.4	13.5	2.8
A6S-3-E2-IR	VERSASSQDNFYDFVVOIR	37.8	30.6	12.0	2.6
	TSEVQRSSQDNFYDFVQAQA	33.1	24.7	9.8	2.5
					0.4

Figure 1E (Cont)

Ratios over Background

Clone	Sequence	Comparisons E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
A6S-4-A6-IGFR	XXXXXXXXXXNFYDMFVXXXX	--	--	--	--	--
A6S-4-E3-IGFR	HVEHMAVQGNFYDMFVQGLR	21	23	--	--	--
A6S-4-D3-IGFR	GRMTGVGRGNFYDMFVQGLR	21	23	--	--	--
A6S-3-E10-IGFR	GLRSEQGNRLNFYDMFVQGLR	20	23	--	--	--
A6S-4-D1-IGFR	RVREKLPENFYDMFVQGLR	23	22	--	--	--
A6S-4-B2-IGFR	SNPSRQDASVNFYDMFVEVLA	22	22	--	--	--
A6S-4-A2-IGFR	QSVDLRSPDSNFYDMFVEVLA	21	22	--	--	--
A6S-4-A5-IGFR	IGGQGHQDGNFYDMFVEVLA	20	22	--	--	--
A6S-4-C1-IGFR	VEVQRHIRKDNFYDMFVQGLD	19	22	--	--	--
A6S-4-B1-IGFR	CWARPCCDAANFYDMFVQGLS	16	22	--	--	--
A6S-4-B4-IGFR	RHERGKEGEGNFYDMFVQGLV	19	21	--	--	--
A6S-4-D4-IGFR	ERSPRPALASNFYDMFVQGLV	19	21	--	--	--
A6S-3-F8-IGFR	IARMRETFQPNFYDMFVDOLA	18	21	--	--	--
A6S-3-H9-IGFR	GRGQGLKRPDNFYDMFVAAAK	25	20	--	--	--
A6S-3-G2-IGFR	YSIEVQDMNENFYDMFVSQGL	23	20	--	--	--
A6S-4-H2-IGFR	TWMEERQDMNFYDMFVQGLK	21	20	--	--	--
A6S-4-A3-IGFR	VTFTSAVFHENFYDMFVRQVS	19	20	--	--	--
A6S-3-G10-IGFR	LAINDLVTHKNFYDMFVDQLR	18	20	--	--	--
A6S-3-E5-IGFR	GAUGLAECPNFYDMFVSQGL	24	19	--	--	--
A6S-3-H2-IGFR	RYRGERHIDGRNFYDMFVEQVN	21	19	--	--	--
A6S-3-G3-IGFR	QGAEGRLSEGNFYDMFVQGLS	21	19	--	--	--
A6S-4-H1-IGFR	PRLIHGSDMGDFYDMFVQGLA	21	18	--	--	--
A6S-4-G1-IGFR	IVAGARHSEVNFYDMFVQGLR	18	18	--	--	--
A6S-4-A1-IGFR	AELVGAGVRGNFYDMFVDQLV	16	16	--	--	--
A6S-2-F1-IGFR	DSRLWLGERNFYDMFVAQIS	17	12	--	--	--
A6S-2-G1-IGFR	VGQGRYVRSNFYDMFVQGLM	30	8	--	--	--
A6S-1-C5-IGFR	RPQLVESGSKNFYDMFVQGLR	30	8	--	--	--
A6S-2-B2-IGFR	RIHNOTERGNFYDMFVHQLV	27	7	--	--	--
	EMYGDTSERVNFYDMFVSAQLQ	30	5	--	--	--

Figure 1F (Con't)

Ratios over Background		Comparisons			
Clone	Sequence	E-Tag	IGFsR	IR	IGFR/IR IR/IGFR
Design	XXXXXXXNFYDFVXXXX	--	--	--	--
A6S-1-D5-IGFR	RVGSGMEDLGNFYDFVVRQAQ	25	5	--	--
A6S-1-A2-IGFR	KDPVTYSQGRNFYDFVVIQ	20	5	--	--
A6S-3-B6-IGFR	DARDHGVWWSNFYDFVQAQVS	20	5	--	--
A6S-1-G3-IGFR	VATVHVGGMNFYDFVQAQVG	19	5	--	--
A6S-3-C4-IGFR	CADPGACSSLNFYDFVQMRG	21	4	--	--
A6S-3-H8-IGFR	NPTSVQYGVNFYDFVNVLS	20	4	--	--
A6S-3-E3-IGFR	RPSLPEVRPGNFYDFVQSVR	19	4	--	--
A6S-3-D9-IGFR	SLQADPFQOCNFYDFVSELA	17	4	--	--
A6S-2-A1-IGFR	LSSRGRTWRNFYDFVQAQVV	31	3	--	--
A6S-1-H4-IGFR	HKSWTTMSPLNFYDFVQAQVE	18	3	--	--
A6S-3-C1-IGFR	RPVIGGGTRNFYDFVQAQMI	17	3	--	--
A6S-3-B10-IGFR	YDQDPYPWGLNFYDFVREVA	16	3	--	--

Figure 1F (Con't)

Clone	Parental/Design	Sequence	Ratios over Background			Comparisons	
			E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
A6L-3-D1-IR		YRGMVLVGRISDAGKVASSEPPARIGQKVFANFYDMFV	19.0	4.0	--	--	--
A6L-4-H7-IR		YRGMVLVGRISDAGKVASSEPPARIGQKVFANFYDMFV	22.6	19.8	26.5	0.7	1.3
A6L-4-H4-IR		YRGMVLVGRISDAGKVASSEPPARIGQKVFANFYDMFV	37.5	3.5	4.2	0.8	1.2
A6L-4-E4-IR		YRGMVLVGRISDAGKVASSEPPARIGQKVFANFYDMFV	38.5	21.1	25.8	0.8	1.2
A6L-4-G7-IR		YRGMVLVGRISDAGKVASSEPPARIGQKVFANFYDMFV	38.1	5.4	6.0	0.9	1.1
A6L-3-C3-IR		YRGMVLVGRISDAGKVASSEPPARIGQKVFANFYDMFV	38.6	16.2	18.5	0.9	1.1
A6L-3-B6-IR		YRGMVLVGRISDAGKVASSEPPARIGQKVFANFYDMFV	34.7	21.8	23.1	0.9	1.1
A6L-4-G11-IR		YRGMVLVGRISDAGKVASSEPPARIGQKVFANFYDMFV	33.1	27.8	30.3	0.9	1.1
A6L-4-G12-IR		YRGMVLVGRISDAGKVASSEPPARIGQKVFANFYDMFV	27.6	2.0	2.0	1.0	1.0
A6L-3-A10-IR		YRGMVLVGRISDAGKVASSEPPARIGQKVFANFYDMFV	32.0	2.3	2.3	1.0	1.0
A6L-4-E12-IR		YRGMVLVGRISDAGKVASSEPPARIGQKVFANFYDMFV	21.1	2.4	2.4	1.0	1.0
A6L-4-E10-IR		YRGMVLVGRISDAGKVASSEPPARIGQKVFANFYDMFV	3.1	2.4	2.4	1.0	1.0
A6L-4-C8-IR		YRGMVLVGRISDAGKVASSEPPARIGQKVFANFYDMFV	30.1	3.8	3.8	1.0	1.0
A6L-3-C12-IR		YRGMVLVGRISDAGKVASSEPPARIGQKVFANFYDMFV	37.9	4.7	4.7	1.0	1.0
A6L-4-H11-IR		YRGMVLVGRISDAGKVASSEPPARIGQKVFANFYDMFV	29.5	5.7	5.7	1.0	1.0
A6L-4-F10-IR		YRGMVLVGRISDAGKVASSEPPARIGQKVFANFYDMFV	35.4	9.6	9.6	1.0	1.0
A6L-4-B9-IR		YRGMVLVGRISDAGKVASSEPPARIGQKVFANFYDMFV	31.6	10.5	10.5	1.0	1.0
A6L-4-H8-IR		YRGMVLVGRISDAGKVASSEPPARIGQKVFANFYDMFV	39.8	12.9	12.9	1.0	1.0
A6L-3-A11-IR		YRGMVLVGRISDAGKVASSEPPARIGQKVFANFYDMFV	38.2	14.6	14.6	1.0	1.0
A6L-4-F9-IR		YRGMVLVGRISDAGKVASSEPPARIGQKVFANFYDMFV	29.0	17.5	17.5	1.0	1.0
A6L-4-G2-IR		YRGMVLVGRISDAGKVASSEPPARIGQKVFANFYDMFV	35.7	18.4	18.4	1.0	1.0
A6L-4-E8-IR		YRGMVLVGRISDAGKVASSEPPARIGQKVFANFYDMFV	29.5	21.4	20.7	1.0	1.0
A6L-4-H10-IR		YRGMVLVGRISDAGKVASSEPPARIGQKVFANFYDMFV	28.7	21.6	21.6	1.0	1.0
A6L-4-G9-IR		YRGMVLVGRISDAGKVASSEPPARIGQKVFANFYDMFV	30.0	22.1	22.1	1.0	1.0
A6L-4-F7-IR		YRGMVLVGRISDAGKVASSEPPARIGQKVFANFYDMFV	37.1	22.6	22.6	1.0	1.0
A6L-4-E11-IR		YRGMVLVGRISDAGKVASSEPPARIGQKVFANFYDMFV	28.6	23.6	24.4	1.0	1.0
A6L-4-E11-IR		YRGMVLVGRISDAGKVASSEPPARIGQKVFANFYDMFV	38.4	26.5	26.5	1.0	1.0

Figure 1G

Clone	Parental/Design	Sequence	Ratios over Background			Comparisons	
			E-Tag	IGFSR	IR	IGFR/IR	IR/IGFR
A6L-4-H9-IR		YRGMVLGRI SDGAGK VASEPPARI GQKVAFNFDWVF	19.0	.4.0	--	--	--
A6L-4-E1-IR		YRMLVLIRI SDAGI VDESEPTTIGQKVAFNFDWVF	37.5	27.3	27.3	1.0	1.0
A6L-3-A5-IR		YRGMVLGRI SDGAGN VASEPSSRI GQKVAFNFDWFI	35.4	32.6	31.4	1.0	1.0
A6L-4-G4-IR		YRGMVLGRI SDGAGK VDEPPARI GQKVAFNFDWVF	38.3	34.6	35.5	1.0	1.0
A6L-4-H2-IR		YRGMVLGRI SDGAGI VASEPPARVQKVAFNFDWVF	30.4	17.7	15.2	1.2	0.9
A6L-4-B6-IR		YRGLTGGRI PDGAGK VASEPPTTIGERVFANFDWVF	36.1	4.2	3.6	1.1	0.9
A6L-4-H5-IR		YRGMVLGRI SDGAGK VAFEPARI GQKVAFNFDWVF	28.6	24.1	22.7	1.1	0.9
A6L-4-H3-IR		YRGMVLGRI SDGAGK VASEPPARI GQKVTFNFDWVF	37.2	24.6	23.1	1.1	0.9
A6L-4-E5-IR		YRGMVLGRI SDGAGK VASDPPAS I GQNVAFNFDWVF	37.1	9.1	7.2	1.3	0.8
A6L-3-CS-IR		YRMLLIDRI SDGASK VSEPPAS I GQKVAFNFDWVF	42.1	30.6	24.4	1.3	0.8
A6L-4-G6-IR		YRMLDLGRI SDGAGK VASEPPARI GQEVYAFNFDWVF	42.2	21.9	17.5	1.2	0.8
A6L-3-D4-IR		YRMLDLGRI SDGAGK VASEPPARI GQKVAFNFDWVF	29.8	4.3	2.8	1.5	0.7
A6L-3-A7-IR		QRGMVLGRI SDGAGK VASEPPARI GQKVAFNFDWVF	39.9	12.4	8.4	1.5	0.7
A6L-3-A6-IR		QRGMVLGRI SDGAGK VDSAPPARI GQKVAFNFDWVF	31.0	21.2	14.0	1.5	0.7
A6L-4-E7-IR		QRGMVLGRI SDGAGK VAFEPARI GQGFAGNFDWVF	25.5	12.3	8.8	1.4	0.7
A6L-3-C6-IR		QRGLVLGRI SDGAGK VASEPPARI GQNVAFNFDWVF	38.4	12.5	7.1	1.7	0.6
A6L-4-F5-IR		QRGMVLGRI SDGAGK VAAEPARI GQKVAFNFDWFI	28.8	10.9	6.7	1.6	0.6
A6L-3-B7-IR		QRGMVLGRI SDGAGK VASEPPARI GQKVAFNFDWFI	33.8	6.3	4.1	1.5	0.6
A6L-4-P4-IR		QRGLVRGRI SDGAGK VSEPPARSGEKVAFNFDWFI	27.6	9.4	5.0	1.9	0.5
A6L-4-E3-IR		QLGMVLGRI SDGSGK VASEPPARI GQKVAFNFDWVF	38.9	17.6	9.4	1.9	0.5
A6L-0-E6-IR		QRGMVLGRI SDGAGK VAFEPARI GQTVFANFDWVF	38.0	6.9	3.8	1.8	0.5
A6L-0-E4-IR		YRGMVLGRI SDGAGH VASEPPARI GQKVAFNFDWVF	31.0	31.0	1.8	17.0	0.1
A6L-0-H3-IR		YRGMVLGRI SDGAGH VASEPPARI GQKVAFNFDWVF	26.0	16.0	1.3	13.0	0.1
		YRGMVLGRI SDGAGK VASEPPARI GQKVAFNFDWVF	27.0	26.0	2.0	13.0	0.1

Figure 1G (Cont.)

Clone	Sequence	Ratios over Background		Comparisons	
Parental/Design		E-Tag	IR	IGFR/IR	IR/IGFR
A6L-4-F8-IGFR	YRGLVLGRISDGAGKVASPPARIGQKVFANFYDMFV	19	4	--	--
A6L-4-F9-IGFR	YRGMVQGRISDGAGKVASPVRRIGQKVFANFYDMFV	26	28	--	--
A6L-2-G9-IGFR	YRGLGLGRISDVAGKVASDPARIGQKVLFPNFYDMFV	39	22	--	--
A6L-4-E7-IGFR	YRGLVLGRISDGAGKVASPPARIGQKVFANFYDMFV	23	22	--	--
A6L-4-G10-IGFR	QGGMLVPGRISDGAGKVASQPPARIGKFGAGNFYDMFV	19	22	--	--
A6L-2-E9-IGFR	YRGMRLVGRISDGAGKVASPPTHIGQKVPNFYDMFV	38	21	--	--
A6L-2-D6-IGFR	YRGLVLGRISDGAGKVASPPARIGQKVPALNFYDMFV	34	21	--	--
A6L-3-H12-IGFR	YRGGWVLGRISDGAGKVASPPGRIGQKVPNFYDMFV	24	21	--	--
A6L-4-A7-IGFR	YRGLGLGRITGGAGKVASPPDRIGQHVDFNFYDMFV	20	20	--	--
A6L-4-A7-IGFR	DGMLVLRISDGAGNVAASEAPRIGQKVFANFYDMFV	20	19	--	--
A6L-4-B8-IGFR	YRGMVRGRISDGAGKAASDPARIGQVLDNFYDMFV	19	19	--	--
A6L-4-G7-IGFR	YRGMVLGRISYAGKVAYPEARMGQKGFANFYDMFV	38	18	--	--
A6L-2-D9-IGFR	YRGLVGGRIAGAGIVASEPPARIGQKVFANFYDMFV	18	18	--	--
A6L-4-F7-IGFR	YRGLGLGRISDGAGKVASPPARMGQKGFANFYDMFV	15	13	--	--
A6L-4-E12-IGFR	YRGLGLGRISAGAGKVASGAPRIGQEDFANFYDMFV	14	13	--	--
A6L-4-H7-IGFR	YRGLMALGRISGAGKVASPPARIGQNVFANFYDMFV	13	12	--	--
A6L-4-H12-IGFR	YRGLMLVGRISDGAGKVASPPARIGQKVLANFYDMFV	17	4	--	--
A6L-2-A4-IGFR	YRGMVPGRIISDGAGGATDPPRIGQKVFANFYDMFV	16	4	--	--
A6L-3-D10-IGFR	YRGLVPGRIISDGAGKVAYPEPARIGQKVFANFYDMFV	15	4	--	--
A6L-2-F6-IGFR	YRGMVPGRIISDGAGKVASPPARIGQKVFANFYDMFV	26	3	--	--
A6L-2-B11-IGFR	YRMLVLRISDGAANVASPPDRIGQKFGAGNFYDMFV	23	3	--	--
A6L-1-B7-IGFR	YRMLALGRISDVTGVASEPFAHIGQKVFANFYDMFV	23	3	--	--
A6L-1-B8-IGFR	YRGMVVRGRIFDGPQKVASPPARIGQKVFANFYDMFV	19	3	--	--
A6L-0-A11-IGFR	YRGLMLGRISDGAGKVASPPARVGDVFNFYDMFV	9	3	--	--
A6L-3-B7-IGFR	YRGLVGGRIISDGAGKVASPPGRIGQKVFANFYDMFV	20	2	--	--
A6L-1-G7-IGFR	QRGLVLRIFDAGKVASDPPARIGQKDFADNFYDMFV	18	2	--	--
A6L-1-B9-IGFR	YRGLMLGRISDGAGKVAPEPPARIGQNVFANFYDMFV	18	2	--	--
A6L-1-C9-IGFR	YRGMVLRISDGAGKVASDPPARIGQKVFANFYDMFV	18	2	--	--
A6L-0-G10-IGFR	YRGLVLRISDGAGKVAEPPASMDSKFGAGNFYDMFV	15	2	--	--
A6L-1-G8-IGFR					

Figure 1H

Clone	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGFSR	IR	IGFR/IR	IR/IGFR	--
E4Dg-1-B8-IR	GFREGNFYDMFVAQVT	40.7	1.0	12.3	0.1	12.3	
E4Dg-1-B8-IR	GFREGQWYFVAQVT	39.6	2.0	1.5	1.3	0.8	
E4Dg-3-E5-IR	GFREGYFYDMFLAQVT	48.7	44.9	31.4	1.4	0.7	
E4Dg-1-A1-IR	GFREGDFYEMFVAQVT	22.9	3.3	2.4	1.4	0.7	
E4Dg-2-D9-IR	GFREGQFYEMFAAQVT	41.8	38.6	26.5	1.5	0.7	
E4Dg-1-B3-IR	GFREGTFYDMFVAQVT	56.3	51.2	32.6	1.6	0.6	
E4Dg-1-A6-IR	GFREGNFYDMFEAQVT	48.9	42.2	26.5	1.6	0.6	
E4Dg-1-A10-IR	GFREGAFYDMFEAQVT	46.9	41.5	26.2	1.6	0.6	
E4Dg-1-A8-IR	GFREGAFYDMFVAQVT	44.1	31.1	19.7	1.6	0.6	
E4Dg-1-B1-IR	GFREGKPYQWFEAQVT	34.0	8.1	4.8	1.7	0.6	
E4Dg-2-C9-IR	GFREGDFYDMFQAAQVT	45.3	40.3	22.5	1.8	0.6	
E4Dg-1-A3-IR	GFREGTFYEMFVAQVT	46.9	41.0	22.5	1.8	0.5	
E4Dg-1-A9-IR	GFREGNFYDMFVAQVT	37.2	14.1	8.0	1.8	0.6	
E4Dg-3-F3-IR	GFREGQFYEMFLAQVT	35.1	16.3	8.7	1.9	0.5	
E4Dg-2-D3-IR	GFREGQFYDMFLAQVT	33.2	5.6	2.8	2.0	0.5	
E4Dg-2-D6-IR	GFREGFYDMFQAAQVT	27.8	4.5	2.3	2.0	0.5	
E4Dg-3-F10-IR	GFREGQFYDMFRAQVT	43.8	23.8	11.4	2.1	0.5	
E4Dg-2-D5-IR	GFREGYFEMFQAAQVT	25.9	7.6	3.7	2.1	0.5	
E4Dg-3-F4-IR	GFREGDFYQWFEAQVT	34.6	4.0	1.9	2.1	0.5	
E4Dg-3-E3-IR	GFREGSFYGMFQAAQVT	20.9	16.0	7.4	2.2	0.5	
E4Dg-3-F8-IR	GFREGSFYAMFQAAQVT	43.1	11.6	5.0	2.3	0.4	
E4Dg-2-C1-IR	GFREGQFYDMFVAQVT	45.3	6.6	2.9	2.3	0.4	
E4Dg-1-B4-IR	GFREGIFYEMFVAQVT						

Figure 11

Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	ICFsR	IR	ICFR/IR IR/GFR
E4D α -4-H5-IR	GFREGNFYDFVAAQVT	47.2	36.0	14.7	2.4 0.4
E4D α -1-B12-IR	GFREGSFYDFVAAQVT	47.6	33.4	13.8	2.4 0.4
E4D α -4-G2-IR	GFREGNFYDFVAAQVT	23.4	20.4	8.6	2.4 0.4
E4D α -3-F9-IR	GFREGSFYDFVAAQVT	36.2	15.6	6.3	2.5 0.4
E4D α -4-G6-IR	GFREGDFYDFVAAQVT	26.0	4.9	2.0	2.5 0.4
E4D α -4-H9-IR	GFREGDFYDFVAAQVT	47.8	24.8	9.5	2.6 0.4
E4D α -2-C10-IR	GFREGGFYDFVAAQVT	42.4	23.2	9.0	2.6 0.4
E4D α -1-B2-IR	GFREGDFYDFVAAQVT	39.4	18.7	7.2	2.6 0.4
E4D α -3-F12-IR	GFREGVFYDFVAAQVT	38.9	16.6	5.6	3.0 0.3
E4D α -2-D11-IR	GFREGGFYDFVAAQVT	40.2	11.1	3.3	3.4 0.3
E4D α -4-H2-IR	GFREGSFYDFVAAQVT	37.8	33.9	8.2	4.1 0.2
E4D β -4-A12-IR	GFREGNFYDFVAAQVT	41.1	8.3	28.7	0.3 3.5
E4D β -4-A10-IR	GFREGKFDYDFVAAQVT	5.8	1.2	2.4	0.5 2.0
E4D β -4-E10-IR	GFREGGFYDFVAAQVT	9.6	1.2	2.2	0.5 1.8
E4D β -4-B11-IR	GFREGTFYDFVAAQVT	36.1	15.2	26.9	0.6 1.8
E4D β -4-C10-IR	GFREGGFYDFVAAQVT	27.8	13.3	23.7	0.6 1.8
E4D β -4-E8-IR	GFREGGFYDFVAAQVT	28.7	16.7	28.2	0.6 1.7
E4D β -4-G7-IR	GFREGGFYDFVAAQVT	30.9	14.7	24.7	0.6 1.7
E4D β -4-C8-IR	GFREGGFYDFVAAQVT	35.5	22.5	32.9	0.7 1.5
E4D β -4-A8-IR	GFREGGFYDFVAAQVT	31.2	14.5	22.2	0.7 1.5
E4D β -4-A9-IR	GFREGGFYDFVAAQVT	35.8	9.0	13.1	0.7 1.4
E4D β -4-G11-IR	GFREGTFYDFVAAQVT	28.9	9.7	13.6	0.7 1.4
E4D β -4-B9-IR	GFREGGFYDFVAAQVT	27.2	9.1	12.5	0.7 1.4
E4D β -4-F10-IR	GFREGGFYDFVAAQVT	7.7	1.5	2.1	0.7 1.4
E4D β -4-D12-IR	GFREGGFYDFVAAQVT	41.1	27.2	36.1	0.8 1.3
E4D β -4-B8-IR	GFREGGFYDFVAAQVT	35.9	27.0	35.2	0.8 1.3
E4D β -4-G10-IR	GFREGAFYDFVAAQVT	38.5	25.5	33.7	0.8 1.3

Figure 11 (Cont)

E4Dβ-4-D9-IR	GFREGFYDWFEAQVT	34.1	19.3	25.7	0.8	1.3
E4Dβ-4-F8-IR	GFREGFYDWFPAQVT	39.3	35.6	44.4	0.8	1.2
E4Dβ-4-E12-IR	GFREGFYDWFDAQVT	40.2	27.8	33.4	0.8	1.2
E4Dβ-4-H12-IR	GFREGAFYDWFPAQVT	41.2	27.1	32.3	0.8	1.2
E4Dβ-4-C9-IR	GFREGQFYDWFPAQVT	38.0	22.5	27.6	0.8	1.2
E4D -4-H9-IR	GFREGNFYDWFPAQVT	38.7	33.3	36.6	0.9	1.1
E4D -4-G9-IR	GFREGDYDWFPAQVT	10.9	4.9	5.6	0.9	1.1
E4Dβ-4-F12-IR	GFREGSFYDWFPAQVT	14.8	5.9	6.1	1.0	1.0
E4Dβ-4-F9-IR	GFREGGFYDWFPAQVT	39.3	31.3	28.3	1.1	0.9
E4Dβ-4-F7-IR	GFREGGFYAWFPAQVT	31.0	22.2	19.5	1.1	0.9
E4Dβ-4-B7-IR	GFREGGFYEW7AQVT	--	--	--	--	--

Figure 11 (Con't)

Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGIsR	IGFR/IR	IR/IGFR
E4D-2-E7-IGFR	GFREGNFYDMFVAQVT	--	--	--	--
E4D-2-C11-IGFR	GFREGGFYDMFVAQVT	20.8	22.8	--	--
E4D-2-B1-IGFR	GFREGGFYDMFVAQVT	21.5	22.6	--	--
E4D-2-D10-IGFR	GFREGGFYDMFVAQVT	22.0	22.5	--	--
E4D-2-A9-IGFR	GFREGGFYDMFVAQVT	20.6	22.1	--	--
E4D-2-E5-IGFR	GFREGGFYDMFVAQVT	17.4	21.5	--	--
E4D-2-H9-IGFR	GFREGGFYDMFVAQVT	24.2	21.2	--	--
E4D-1B-C4-IGFR	GFREGGFYDMFVAQVT	19.1	20.7	--	--
E4D-2-E10-IGFR	GFREGGFYDMFVAQVT	24.3	20.5	--	--
E4D-2-F4-IGFR	GFREGNFYDMFVAQVT	21.0	20.5	--	--
E4D-2-C10-IGFR	GFREGGFYDMFVAQVT	25.0	20.2	--	--
E4D-3-D8-IGFR	GFREGGFYDMFVAQVT	22.8	20.1	--	--
E4D-3-F9-IGFR	GFREGGFYDMFVAQVT	21.1	19.8	--	--
E4D-1B-E5-IGFR	GFREGGFYDMFVAQVT	22.6	19.7	--	--
E4D-2-F3-IGFR	GFREGGFYDMFVAQVT	24.2	18.8	--	--
E4D-3-D5-IGFR	GFREGGFYDMFVAQVT	23.6	18.0	--	--
E4D-3-G10-IGFR	GFREGGFYDMFVAQVT	22.2	18.0	--	--
E4D-2-F6-IGFR	GFREGGFYDMFVAQVT	22.1	17.6	--	--
E4D-2-F7-IGFR	GFREGGFYDMFVAQVT	24.6	17.5	--	--
E4D-3-B7-IGFR	GFREGNFYDMFVAQVT	19.0	17.5	--	--
E4D-1B-C12-IGFR	GFREGGFYDMFVAQVT	23.0	16.4	--	--
E4D-3-B1-IGFR	GFREGGFYDMFVAQVT	23.0	16.1	--	--
E4D-2-E2-IGFR	GFREGGFYDMFVAQVT	21.6	16.0	--	--
E4D-2-D1-IGFR	GFREGGFYDMFVAQVT	21.9	14.1	--	--
E4D-1-D4-IGFR	GFREGGFYDMFVAQVT	24.5	13.2	--	--
E4D-1B-A10-IGFR	GFREGGFYDMFVAQVT	18.9	12.4	--	--
E4D-1B-A3-IGFR	GFREGGFYDMFVAQVT	23.9	10.8	--	--
E4D-1-B5-IGFR	GFREGGFYDMFVAQVT	22.2	10.8	--	--
E4D-1-B5-IGFR	GFREGGFYDMFVAQVT	19.0	10.8	--	--

Figure 1J

Clone	Design	Sequence	Ratios over Background			Comparisons		
			E-Tag	IGFSR	IR	IGFR/IR	IR/IGFR	
		XXXXXXXXXHFNFYDMFVRQVSSXXXXX	--	--	--	--	--	
Parental		VTFTSAVHNFNFYDMFVRQV	29.8	17.5	16.3	1.1	0.9	
H2CA-4-F11-IR		TYKARFLHNFYDMFNQVQSYFGRV	37.7	2.2	18.1	0.1	8.2	
H2CA-4-B10-IR		QKSLHHEQFYDMFVRQVSLGAGG	31.2	4.4	18.8	0.2	4.3	
H2CA-4-G3-IR		GGKVNPFHEDFYDMFVRQVSGVSDR	36.1	13.4	25.7	0.5	1.9	
H2CA-3-A11-IR		LVGAPAFHEDFYDMFVRQVSGCCQEQ	35.6	12.1	22.0	0.5	1.8	
H2CA-4-F8-IR		TGAEVSPHNFYDMFDRQVSSWLDSD	36.0	21.1	33.5	0.6	1.6	
H2CA-4-Q4-IR		QPHSSRLHNFYDMFDRQVFWYALDR	37.1	23.3	34.3	0.7	1.5	
H2CA-4-F4-IR		SRALAAVHEQFYDMFVRQVSLDMGY	39.8	25.0	35.6	0.7	1.4	
H2CA-4-H10-IR		QPKDGTLHNFYDMFVRQVSSGWG	33.5	5.1	6.6	0.8	1.3	
H2CA-4-F1-IR		RGRLIQHEDFYDMFLRQVSGMGGS	36.1	19.6	25.1	0.8	1.3	
H2CA-3-D5-IR		QRGAPKSDNFYDMFVRQVLRFGEND	39.3	24.3	31.9	0.8	1.3	
H2CA-4-E11-IR		AARTSLFHEQFYDMFVRQVSGCMG	8.2	2.6	3.2	0.8	1.2	
H2CA-3-B6-IR		GTSNHSLNHNFYDMFVRQVSSVSSG	35.9	9.9	12.1	0.8	1.2	
H2CA-4-F1-IR		VSHVHLHNFYDMFVRQVLAAGFSG	37.3	30.1	36.2	0.8	1.2	
H2CA-4-H5-IR		GRQDSGLHGFYDMFVRQVQVEALG	38.6	35.4	37.3	1.0	1.1	
H2CA-3-C9-IR		SNDRQFHEQFYDMFVRQVSGADGR	29.3	5.1	5.6	0.9	1.1	
H2CA-3-A10-IR		LSTEQRPHEKFYDMFVRQVSSGGGT	37.2	16.9	19.1	0.9	1.1	
H2CA-3-A3-IR		SLSRQFHEHNFYDMFVRQVSELEGV	29.2	28.6	32.2	0.9	1.1	
H2CA-4-C8-IR		IPGRRSLHNFYDMFVRQVSPGGGA	32.4	29.1	31.6	0.9	1.1	
H2CA-4-G9-IR		TQKAQSLDEKFYDMFVRQVSGGLTG	36.1	34.4	36.4	0.9	1.1	
H2CA-4-G10-IR		VQSLSDFHNFYDMFVRQVQIAGAEWT	34.2	35.5	37.7	0.9	1.1	
H2CA-4-H7-IR		NGTSQALHQNFYDMFVRQVSGEPGP	37.0	36.0	40.0	0.9	1.1	
H2CA-4-F9-IR		VQSVTFHGFYDMFVRQVSGQFEG	37.5	36.7	39.5	0.9	1.1	
H2CA-4-F7-IR		TIDHPLHEQFYDMFVRQVSDLESIG	37.7	37.6	39.9	0.9	1.1	
H2CA-3-D10-IR		PVNGYAFHNFYDMFVRQVSIIEKAG	18.7	3.6	3.5	1.0	1.0	
H2CA-3-B1-IR		SRGSGVFHESFYDMFVRQVSEWTFQF	26.5	21.4	21.5	1.0	1.0	
H2CA-3-A5-IR		QPVSGSVHREFYDMFVRQVSGAGG	32.9	22.9	22.4	1.0	1.0	
H2CA-4-F10-IR		ASQLPVPYENFYDMFVRQVSLDAQRE	26.6	27.7	28.5	1.0	1.0	

Figure 1K

Clone	Design	Sequence	Ratio over Background		Comparisons	
			E-Tag	IGFR	IGFR/IR	IR/IGFR
H2CN-3-D12-IR		XXXXXXSHENTDMFVQVQSGGALRG	36.8	34.1	29.6	1.2
H2CN-3-B5-IR		WYGRISTHENTDMFVQVQSGATTC	29.8	35.2	30.5	1.2
H2CN-4-E1-IR		GVVRQPHQFTDMFVQVQSGEGDA	29.8	12.5	11.3	0.9
H2CN-4-E3-IR		PDABQPHFTDMFVQVQSGEGANS	33.1	29.9	27.5	1.1
H2CN-4-E12-IR		FGVGHCHENTDMFVQVQSGGALRG	33.3	32.3	30.2	1.1
H2CN-3-A6-IR		ETPLTLHJGFDMFVQVQSGGALRG	36.0	32.4	29.4	1.1
H2CN-4-E8-IR		GVVRQPHQFTDMFVQVQSGEGDA	34.0	32.1	30.6	1.1
H2CN-4-E3-IR		PDABQPHFTDMFVQVQSGEGANS	36.8	33.7	29.7	1.1
H2CN-4-H6-IR		QVAVQCNDFTAMFAQVVEDPFAVA	41.0	34.2	32.0	1.1
H2CN-4-H2-IR		RNNLQPHENTDMFVQVQSGALRG	37.1	34.5	30.8	1.1
H2CN-3-D4-IR		REBQVPHENTDMFVQVQSGGALRG	41.8	35.3	32.8	1.1
H2CN-3-D1-IR		GVVRQPHQFTDMFVQVQSGEGDA	38.7	32.5	31.3	1.1
H2CN-3-D8-IR		GVVRQPHQFTDMFVQVQSGEGDA	34.5	32.3	31.3	1.1
H2CN-3-C1-IR		QVAVQCNDFTAMFAQVVEDPFAVA	39.9	36.7	33.9	1.1
H2CN-3-D8-IR		GVVRQPHQFTDMFVQVQSGEGDA	37.8	36.7	33.1	1.1
H2CN-4-H4-IR		QVAVQCNDFTAMFAQVVEDPFAVA	38.5	37.0	33.7	1.1
H2CN-4-F6-IR		VFVRSCHINTDMFVQVQSGQDQ	34.7	37.5	35.2	1.1
H2CN-4-E4-IR		LLASRSHENTDMFVQVQSGATTC	33.6	38.0	34.7	1.1
H2CN-3-C1-IR		QVAVQCNDFTAMFAQVVEDPFAVA	40.3	38.1	35.1	1.1
H2CN-3-C4-IR		ANQDQPHENTDMFVQVQSGEGDA	39.4	38.3	35.1	1.1
H2CN-3-D7-IR		QVAVQCNDFTAMFAQVVEDPFAVA	39.3	38.8	35.8	1.1
H2CN-3-A7-IR		QVAVQCNDFTAMFAQVVEDPFAVA	42.5	39.2	35.5	1.1
H2CN-4-G12-IR		GVAVQCNDFTAMFAQVVEDPFAVA	35.3	35.2	31.6	1.3
H2CN-3-D6-IR		QVAVQCNDFTAMFAQVVEDPFAVA	37.6	36.0	32.2	1.3
H2CN-4-H12-IR		DRPSTHENTDMFVQVQSGGALRG	39.4	38.2	35.3	1.3
H2CN-4-H12-IR		REBQVPHENTDMFVQVQSGGALRG	38.1	37.9	32.9	1.2
H2CN-3-C12-IR		POBRLSHENTDMFVQVQSGALRG	38.5	38.4	31.7	1.2

Figure 1K (Con't)

Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFR IR	IGFR/IR	IR/IGFR
H2CA-4-G7-1R	XXXXXXXXPHENFYDNFVRQVSXXXXXX	--	--	--	--
H2CA-3-C6-1R	RAGGVGLHDNFYDNFVRQVSGDSDGP	35.9	34.7	23.7	1.5
H2CA-3-B8-1R	ADCYVQLHENFYDNFVRQVCNLQEGM	38.7	37.6	28.2	1.3
	RQGHAGPHDNFYDNFVRQVSGSTPQV	37.8	19.6	9.9	2.0
					0.5

Figure 1K (Con't)

Clone	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGFSR	IR	IGFR/IR	IR/IGFR	--
Parental	XXXXXXPHENFYDMFVQVQSXXXXX	29.8	17.5	16.3	1.1	0.9	--
H2CA-4-G9-IGFR	VTFTSVAFHNFYDMFVQVQS	8.6	9.5	0.6	16.0	0.1	0.1
H2CA-4-H6-IGFR	GI1SQSPESFYDMFAGQVSDPMCM	4.9	10.5	0.7	14.6	0.1	0.1
H2CA-4-F-IGFR5	VGRASGFPEFYDMFGRQLSLGSEQ	5.5	9.7	0.8	12.3	0.1	0.1
H2CA-4-H8-IGFR	VGYQQGDHNFYDMFIRQVSGRLVQ	5.6	9.2	1.0	9.4	0.1	0.1
H2CA-4-F11-IGFR	SACQDCHEFYDMFARQVSGGAAYG	3.5	6.8	1.0	6.7	0.1	0.1
H2CA-4-F6-IGFR	SAAQLFFQESFYDMFLRQVAESQPN	3.9	7.3	1.1	6.4	0.2	0.1
H2CA-4-F10-IGFR	AVRATRFDEAFYDMFVRQLSDQGNK	4.9	5.7	1.0	5.9	0.2	0.2
H2CA-1-A3-IGFR	VNQSGLIHENFYDMFROVSHORGVR	7.7	3.8	0.8	5.1	0.2	0.2
H2CA-3-C8-IGFR	APDPSDFQELFYDMFVRQVSRMPGG	15.1	5.6	1.2	4.8	0.2	0.2
H2CA-2-B9-IGFR	SSCDGAGHESFYEMFVRQVSGCRSV	9.3	7.0	1.7	4.2	0.2	0.2
H2CA-4-H4-IGFR	RAGSSDFHEDFYEMFVRQVSLKKG	3.9	4.1	1.0	4.2	0.2	0.2
H2CA-4-F7-IGFR	QAVQGFHEEFYDMFVRQVSTGVGG	1.5	3.2	0.8	4.1	0.2	0.2
H2CA-3-D6-IGFR	SSIGGGPHENFYDMFGRQLSOSPLK	8.3	9.0	2.2	4.0	0.3	0.3
H2CA-3-D8-IGFR	QSPVGSSEHEDFYDMFRRQVQAQGAHQ	10.9	7.2	1.8	4.0	0.3	0.3
H2CA-4-G11-IGFR	NYRRQWFNGNFYDMFDRQVSLVTFG	10.8	9.5	2.5	3.9	0.3	0.3
H2CA-4-F1-IGFR	TLDGSGSEQFYDMFVRQLSRTNPD	5.8	3.5	0.9	3.8	0.3	0.3
H2CA-3-D7-IGFR	FYVQMGHENFYDMFDRQVSGSGAG	13.3	3.0	0.8	3.7	0.3	0.3
H2CA-1-A7-IGFR	LARQA PVEENFYDMFVRQVSGDRVGG	8.0	2.2	0.6	3.7	0.3	0.3
H2CA-2-B4-IGFR	RCREL VHSFTFYDMFDRQVAGRTCP	3.5	4.1	1.1	3.6	0.3	0.3
H2CA-2-B3-IGFR	CCLLCRFQNFYDMFVQCGLSLRPL	7.7	3.8	1.0	3.6	0.3	0.3
H2CA-2-B2-IGFR	PLASDLQVQFYDMFVRQVSPGRGG	4.1	3.4	1.0	3.5	0.3	0.3
H2CA-3-D4-IGFR	GAPVDQLHEDFYDMFVRQVQAATG	17.6	13.8	4.1	3.4	0.3	0.3
H2CA-4-F2-IGFR	RSAGSLPQOFYDMFVRQVLSGTDK	9.3	12.8	4.2	3.0	0.3	0.3
H2CA-3-D11-IGFR	SRVTTVHENFYDMFVRQLSDSAISG	12.2	6.9	2.3	3.0	0.3	0.3
H2CA-4-H9-IGFR	DERGKRFEDFYDMFVRQVSESRFGQ	8.7	5.6	1.9	3.0	0.3	0.3
H2CA-2-B11-IGFR	RCVAGPHQFYDMFDRQVSRVHKFG	11.9	4.6	1.6	3.0	0.3	0.3
H2CA-3-E8-IGFR	AICDAGPHHFFYDMFALQVSDCGRQS	13.2	6.3	2.2	2.9	0.3	0.3
	LGYYEPFPQNFYDMFVRQVSGAENAG						

Figure 1L

Clone	Sequence	Ratios over Background		Comparisons	
Design		E-Tag	IGFsR	IGFR/IR	IR/IGFR
H2CA-3-E6-IGFR	XXXXXXXXXXXXXXXXXXXX	--	--	--	--
H2CA-4-F4-IGFR	WRGHGTFHEDFYDMFVRQVSGSGST	15.7	8.7	3.1	2.8
H2CA-3-D10-IGFR	GGKGVGLHNFYDMFDRQVSLGADG	11.5	7.4	3.0	2.5
H2CA-3-E1-IGFR	CNLTAGFHEQFYHMFALQVCGDAENA	9.4	6.8	2.9	2.3
H2CA-2-B6-IGFR	ERGEDMFHNFYDMFVRQISGRGGG	12.5	6.4	2.8	2.3
H2CA-3-E11-IGFR	TNQGVSFYDSFYGMFVRQIQGVDSG	18.0	6.2	2.7	2.3
H2CA-4-H2-IGFR	HLADGQFHEKFDYDMFERQISSRCNDC	4.7	2.2	1.0	2.2
H2CA-3-C11-IGFR	QTFGKSLHNFYDMFVRQVSGREGGD	9.8	9.9	4.8	2.1
H2CA-2-B8-IGFR	FRTLAHQHDSFYDMFDRQVSGAAGER	9.3	3.3	1.6	2.1
	SASTHGFHNFYDMFVRQVSGAQKIL	14.6	7.9	3.9	2.0

Figure 1L (Con't)

Clone	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR	--
Parental	XXXXXXXXXXFYWFYXXXXX	29.8	17.5	16.3	1.1	0.9	
H2CBa-3-B12-1R	VFTSAVFHENFYDFVFRQVS	26.0	1.3	20.4	0.1	16.0	
H2CBa-3-D2-1R	QSDSGTVHDFRYGWFPRDT*A	20.6	1.7	12.1	0.1	7.0	
H2CBa-3-D2-1R	WTDVDGFHSFYWFNFQWNER	24.6	2.1	14.0	0.1	6.7	
H2CBa-3-D12-1R	VASGHV LHGQFYWFVDOFAL	16.7	2.4	15.1	0.2	6.3	
H2CBa-3-H5-1R	QARVNGHQQFYWFNFWVG	31.4	2.5	13.9	0.2	5.6	
H2CBa-3-B6-1R	VGDGFCVSHDQFYWFLRESMQ	22.7	1.4	6.4	0.2	4.7	
H2CBa-3-G11-1R	SGSRPFVHEGQFYWFVDOOG	25.9	1.7	7.1	0.2	4.3	
H2CBa-3-A6-1R	QFSAGAFHGDFYGFWMFALYNG	33.4	6.0	25.5	0.2	4.3	
H2CBa-3-B1-1R	SRDEFLHHQFYWFVRLNLEP	23.0	4.8	19.8	0.2	4.1	
H2CBa-3-F8-1R	DSVNSDLHRAFYGWFAEQWRA	14.0	2.2	8.5	0.3	4.0	
H2CBa-3-E11-1R	GSVDREBIGHQFYWFSFEQLMG	24.9	2.2	6.9	0.3	3.2	
H2CBa-3-G4-1R	SAKTPVLHGGFYWFMFAQSES	23.6	2.6	8.0	0.3	3.1	
H2CBa-3-D3-1R	LVVGRFRHQSFYDFVFAAAGG	27.0	5.6	16.4	0.3	2.9	
H2CBa-3-C1-1R	IMWCFITQDFFYCFWQFTEQGR	23.3	1.1	3.1	0.4	2.8	
H2CBa-3-C3-1R	VVGFPLDIHREFYGFHQQQGA	23.7	6.7	17.6	0.4	2.6	
H2CBa-3-G3-1R	VVPKAGGHEAFYWFRRQDGD	28.8	8.3	21.9	0.4	2.6	
H2CBa-3-B4-1R	QSFVTSVHTHRTFYAMFASALBM	26.7	7.0	17.2	0.4	2.5	
H2CBa-3-G5-1R	SRGLGVHSGFYWFEXQFNFQ	28.0	8.6	19.4	0.4	2.3	
H2CBa-3-B11-1R	GADTGAVHRRFYWLFPEQLSGG	31.3	11.3	24.9	0.5	2.2	
H2CBa-3-A1-1R	PGNRPTFHAEFYWFRFAQGS	27.2	10.6	23.9	0.4	2.2	
H2CBa-3-H1-1R	VAVANGLHESFYAMFNQFSD	21.1	6.1	12.7	0.5	2.1	
H2CBa-3-F12-1R	GFNTGTTFHDQFYWFWEAAGG	21.0	9.7	19.1	0.5	2.0	
H2CBa-3-H7-1R	GDGLTAPHQGFYWFEDIQMG	26.0	12.7	24.7	0.5	1.9	
H2CBa-3-C12-1R	VGVNRQFHTFRFYAMFDEQLGG						

Figure 1M

Clone	Design	Sequence	Ratios over Background		Comparisons		
			E-Tag	IR	IGFR/IR	IR/IGFR	
H2CBα-3-D11-IR		XXXXXXFXHXXFXWFXFXXXXXX	27.8	13.0	24.8	0.5	1.9
H2CBα-3-H12-IR		GPRQRLHDAFYSNFQALRVN	27.4	7.2	12.4	0.6	1.7
H2CBα-3-A10-IR		LGTIAVHELFXWFERQLGG	27.1	13.2	22.3	0.6	1.7
H2CBα-3-A5-IR		LGVCYGCNCQYRWFNDLADR	28.3	16.1	28.1	0.6	1.7
H2CBα-3-C4-IR		FSGWADYQSGFYQWFAEELAN	30.7	17.2	29.2	0.6	1.7
H2CBα-3-B8-IR		WGPFSVFDSEFYRWFQAASDD	25.6	11.3	18.6	0.6	1.6
H2CBα-3-H11-IR		PNNEGLVHGLFYDMFQALSG	28.8	14.0	22.4	0.6	1.6
H2CBα-3-E10-IR		DEGGAPLDVNFYRWFQAVRG	27.7	14.3	23.0	0.6	1.6
H2CBα-3-C2-IR		QSGNFGSHGAFYSNFDRVLAN	28.4	17.0	26.7	0.6	1.6
H2CBα-3-F6-IR		MQRDGFNSFYGNFMAALGE	27.3	14.5	21.8	0.7	1.5
H2CBα-3-D4-IR		SPERKKVHSQFYSNFDRQLLG	29.0	18.9	27.1	0.7	1.4
H2CBα-3-A7-IR		PSNAPFHGGFYDMFDMVQGS	29.1	19.4	26.9	0.7	1.4
H2CBα-3-H4-IR		PHRPGSFNTNFYQWFDQMNQ	27.2	20.1	27.9	0.7	1.4
H2CBα-3-B7-IR		SDSSSTLNGRFYTWFMQLLD	28.6	18.0	23.6	0.8	1.3
H2CBα-3-F9-IR		QRGGGFHEGFYSWFRSQSL	26.1	19.1	24.3	0.8	1.3
H2CBα-3-H6-IR		SGSRPVFHEQFYEWFDQLGL	24.8	21.6	27.3	0.8	1.3
H2CBα-3-F5-IR		GGSSQAFHCAFYFWFSAQLRG	29.4	22.0	27.8	0.8	1.3
H2CBα-3-A2-IR		AFVSEKVRQRFYDMFDMQMS	30.7	22.5	29.1	0.8	1.3
H2CBα-3-F3-IR		VRHPTRFHDEFYRWFTEQLTT	16.3	6.7	9.0	0.7	1.2
H2CBα-3-G6-IR		ARLLNIFDRGYNWFORQLDE	24.9	21.0	24.4	0.9	1.2
H2CBα-3-G7-IR		PSLSSNHSFYRWFQVLVST	24.4	18.7	23.0	0.8	1.2
H2CBα-3-C5-IR		FAFLGLGHQCFYDMFQALLEG	26.4	21.2	25.4	0.8	1.2
H2CBα-3-G1-IR		VSATVMLHREFYDMFQGLLD	26.9	21.5	26.3	0.8	1.1
H2CBα-3-E3-IR		GGVSGVILHDFYSNFQALAG	24.2	17.2	19.3	0.9	1.1
H2CBα-3-E3-1R		GLGIASFHEGFYSNFTAQLAG					

Figure 1M (Cont)

Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IR	ICFR/IR	IR/ICFR
H2CBa-3-A9-IR	XXXXXXXXXXFYWFXXXXX	30.5	21.7	24.1	0.9
H2CBa-3-C11-IR	RVDAALNAGFYWFRGVIQG	26.4	21.8	23.2	0.9
H2CBa-3-B4-IR	EGARQGFHARFYWFAQJLAL	30.9	22.0	24.3	0.9
H2CBa-3-F11-IR	VLLGVVHGGFYDMFSRQLSS	24.5	22.5	23.9	0.9
H2CBa-3-G10-IR	GALSDRYNNVYDMFREQLLG	28.3	23.6	27.1	0.9
H2CBa-3-B2-IR	PDSFMSLHORFYWFAQVGT	31.4	23.6	25.3	0.9
H2CBa-3-C7-IR	RVYKXNPHNFYWFREQLLG	26.8	24.0	25.7	0.9
H2CBa-3-B5-IR	ABLLERQDPFYWFETLMDG	28.7	25.0	26.4	0.9
H2CBa-3-G9-IR	HSGMRDVHARFYWFSQJSG	30.0	25.2	28.7	0.9
H2CBa-3-A12-IR	RNSSGNFHKFYWFAQJLKG	27.8	25.2	26.7	0.9
H2CBa-3-C9-IR	GSMSPVNDQFYWFRDLVDE	28.0	26.4	28.7	0.9
H2CBa-3-B10-IR	CTGRQFDGCFYWFEDQLVG	32.1	28.7	31.9	0.9
H2CBa-3-E1-IR	GIAVQSLHDSFYRWFNALGS	33.5	30.8	33.2	0.9
H2CBa-3-G12-IR	IGPPGSLHRGFDYWFARQVEA	31.7	30.5	29.0	1.1
H2CBa-3-F7-IR	GAAGISFHRGFDYWFARQVRD	29.1	31.4	29.8	1.1
H2CBa-3-G8-IR	GVDVTDPHKDFYSWFQRLNG	23.2	20.7	20.3	1.0
H2CBa-3-C6-IR	WAGRAGIHGGFYWFENRQLRG	22.8	20.9	20.4	1.0
H2CBa-3-H8-IR	LSQLAAPHLGFYWFSEVAA	26.7	21.2	22.0	1.0
H2CBa-3-F2-IR	VHSVRLNVGFYWFQDQLSG	23.4	22.5	22.0	1.0
H2CBa-3-D5-IR	LGLMALFDRGFYWFPEQQLSG	23.5	23.4	23.2	1.0
H2CBa-3-F10-IR	VARGSSLHDDFYWFASQLRT	25.5	24.3	25.2	1.0
H2CBa-3-D10-IR	LGYYGALNTQFYWFAQLVGS	26.7	24.5	25.6	1.0
H2CBa-3-F10-IR	EDSLRLHGGFYWFRKQLGD	26.8	24.9	24.9	1.0
	GRDNMKFHSFYWDFQQLAG	25.7	25.6	26.1	1.0

Figure 1M (Cont'l)

Clone	Design	Sequence	Ratios over Background		Comparisons	
			E-Tag	IR	IGFR/IR	IR/IGFR
H2CB α -3-D6-IR		XXXXXXFHHXXFYXWFXXXXXX	27.9	26.0	25.8	1.0
H2CB α -3-H3-IR		AGVMGGFHQEFYLMFERALSN	27.0	26.9	26.2	1.0
H2CB α -3-F4-IR		AGHVQVYDGYGFWFREQLGA	31.2	27.2	27.7	1.0
H2CB α -3-E9-IR		FVQNIIGDYDFYGMFVREVEK	31.6	27.7	28.2	1.0
H2CB α -3-H10-IR		PVGIIGLHRAFYQWFQSQVDA	26.9	27.9	28.8	1.0
H2CB α -3-G2-IR		GSRQADHQAFYDWFNLVLGV	29.1	28.1	28.8	1.0
H2CB α -3-B2-IR		AGGRKPHDDFYGMFRDQLAE	29.4	28.1	28.2	1.0
H2CB α -3-E8-IR		DLASHGHDAFYNWFVQLNS	31.5	28.4	29.1	1.0
H2CB α -3-E5-IR		GSNGGVHQQFYAMFEALSG	33.0	28.7	28.9	1.0
H2CB α -3-E6-IR		RGRASTFHDDFYGMFSQQLRF	29.6	29.0	28.1	1.0
H2CB α -3-E7-IR		SPARRVSHDDFYGMFAKQLES	30.4	30.2	30.2	1.0
H2CB α -3-C8-IR		SSDVGAFFHSFADMFKAQLSG	31.9	31.2	31.5	1.0
H2CB α -3-A4-IR		PTVHFAFDLIFYGMFAKQVED	32.2	31.9	32.6	1.0
H2CB α -3-D1-IR		SNTVTGLDERFYAMFEVQLGA	32.9	32.5	31.5	1.0
H2CB α -3-B9-IR		PGAAFGFHSAFYDMFPAQAVSG	33.2	33.8	33.3	1.0
H2CB α -3-D8-IR		MRSEASFHVEFYSNFEQLRS	26.3	20.2	19.1	1.1
H2CB α -3-F1-IR		VSRYGQDQGFYHWFSDLLKG	28.8	28.0	26.4	1.1
H2CB α -3-A11-IR		RPSSGGLHYGFYHWFVQBEM	20.5	21.5	17.7	1.2
H2CB α -3-A3-IR		SNTIEHFHMQFYHWFSDALGN	30.4	29.6	21.8	1.4
		ANDCLGLHAGFYGMFAQCQLGG				

Figure 1M (Cont'd)

Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR/IGFR
	XXXXXXXXXXFXFXFXFXFX	--	--	--	--
H2CB β -3-E8-IR	TGHRGLDGEQFYWFRDALSG	15.9	1.9	11.8	0.2
H2CB β -4-F8-IR	VLTSTNLHQRFYSWFAARRE	13.4	0.8	2.6	0.3
H2CB β -3-C4-IR	CVAQGGFQSSFYCWFAGLDID	21.1	1.3	4.0	0.3
H2CB β -3-D5-IR	NGQSSRFHTAFYDWFAQLSG	14.0	3.3	10.2	0.3
H2CB β -3-E6-IR	SVPRGTVDHAFYQWFEVALG	5.7	0.7	2.1	0.3
H2CB β -4-G12-IR	GARGSTPHDQFYWFWQLGD	6.8	1.8	5.4	0.3
H2CB β -4-F4-IR	PPGMNGHTSFYSWFVDQLGD	17.9	1.9	5.6	0.3
H2CB β -4-F11-IR	AVCTLYHSQFYRWFRQLGG	15.0	1.7	4.8	0.3
H2CB β -3-E5-IR	ELQARGVHRNFYRWFEAVSG	17.0	1.8	5.0	0.4
H2CB β -4-F2-IR	HRVARAFHEQFYDWFEKAVSG	15.9	1.3	3.4	0.4
H2CB β -4-G4-IR	GAMEPDYHRSFYQWFAALGE	8.7	1.4	3.5	0.4
H2CB β -3-C8-IR	CPDRQSDDRFYWNFADALAS	4.9	1.4	3.2	0.4
H2CB β -4-F10-IR	GGAQISFHERFYQWFLQEAAG	10.2	1.0	2.4	0.4
H2CB β -4-H4-IR	HKRGIVQHGFAYFWFDSLLSG	20.8	4.2	9.5	0.4
H2CB β -4-G6-IR	QASDNRSDGQFYLWFPEKLLS	14.5	5.6	8.5	0.7
H2CB β -4-H1-IR	DRGRMGVDEGFYWNFAQMQE	17.0	10.1	13.2	0.8

Figure 1M (Cont)

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	ICF5s	IR	ICGFR/IR	IR/ICGFR
Parental	XXXXXXXXXXFXFXFXFX	--	--	--	--	--
H2CB-3-D2-IGFR	VTFSTAVHNFVDFVRQVS	29.8	17.5	16.3	1.1	0.9
H2CB-3-C12-IGFR	TASQECDDGFFVGMFRMKT	22.9	18.6	11.8	1.6	0.6
H2CB-3-B11-IGFR	SIDMRSEFPFRWFQALAG	17.3	19.6	13.0	1.5	0.7
H2CB-4-E2-IGFR	CMSLSDCHRRKFGWFKSQGGE	24.6	17.1	11.9	1.4	0.7
H2CB-3-A5-IGFR	LALCRSPGSGFYGMFAAVGC	22.4	21.0	16.5	1.3	0.8
H2CB-4-G12-IGFR	PRSATNSDGSFYWFAAQLGL	28.8	26.1	22.6	1.2	0.9
H2CB-3-B2-IGFR	LRRSSVPHDPYE*ISRLLVGS	23.7	23.8	19.4	1.2	0.8
H2CB-3-D1-IGFR	ARLQQPHGGFYENFRAQVSP	23.0	19.9	16.4	1.2	0.8
H2CB-3-B6-IGFR	AQLNLNLCHEFFYWFCAVTR	21.5	19.5	15.7	1.2	0.8
H2CB-4-F7-IGFR	WTCDTAFHQQFYQWFCDKLV	16.3	4.5	3.7	1.2	0.8
H2CB-4-G8-IGFR	GKEGFGILDRFYWMFREQLGP	22.0	19.0	18.0	1.1	0.9
H2CB-3-D4-IGFR	GRAPSFDCDFYCFWRNQVS	20.2	18.6	16.5	1.1	0.9
H2CB-3-D5-IGFR	DVEAETQHLRFYAMFSLQGS	21.9	18.3	16.9	1.1	0.9
H2CB-4-E6-IGFR	TSVTAVFHGGFYGMENQVSK	21.4	17.9	16.4	1.1	0.9
H2CB-3-C2-IGFR	NSEHGRLLVDVFGWFRVLIQQ	19.6	15.8	14.8	1.1	0.9
H2CB-3-A6-IGFR	GPLDGCCQGGFYGMFCQVST	18.8	12.2	10.8	1.1	0.9
H2CB-4-H12-IGFR	KRSAYNFPDFYDMFRMQLSG	26.8	29.0	28.1	1.0	1.0
H2CB-3-B10-IGFR	ASEPGYLDPFYGMFREQLEA	23.9	28.3	28.1	1.0	1.0
H2CB-4-F11-IGFR	NRGDSGVHSFYGMFRLQLSG	27.1	27.5	27.3	1.0	1.0
H2CB-4-G11-IGFR	ASKGSSILHNDFYGMFAQQLAR	25.5	25.5	24.6	1.0	1.0
H2CB-4-E12-IGFR	ANVSMWITQGVYDMFDAQLRQ	25.3	25.4	25.3	1.0	1.0
H2CB-4-G10-IGFR	RTSPGSLHDPFYDMFQQQGG	27.8	24.9	24.7	1.0	1.0
H2CB-3-B9-IGFR	POVMSSPHGGFYWMFREQLNG	25.1	24.6	24.2	1.0	1.0
H2CB-3-B7-IGFR	CIANSEDDSGSYGMFALQGS	25.6	23.3	23.7	1.0	1.0
H2CB-4-H4-IGFR	GGSMGMHDSFYGMFALQLRS	24.0	23.2	23.5	1.0	1.0
	RPQGGSIHAGFYQWFRDAVAG	23.5	23.1	23.8	1.0	1.0

Figure 1N

Clone	Design	Sequence	Ratios over Background		Comparisons	
			E-Tag	IR	IGFR/R	IR/IGFR
H2CB-4-H10-IGFR		XXXXXXHXXFXFXFXFXFXFX	--	--	--	--
H2CB-4-H5-IGFR		GALSSLFDAFYGFYFNRLQEG	21.9	22.4	23.3	1.0
H2CB-4-F4-IGFR		KVDLRGFHGFYGFYFNRLQAG	22.3	22.3	21.6	1.0
H2CB-4-F4-IGFR		CSGLQRCHDSFYGFYFNRLQAG	23.1	21.6	20.6	1.0
H2CB-3-D8-IGFR		DSLGIHFHGFYGFYFNRLQDM	21.3	20.9	21.3	1.0
H2CB-4-E4-IGFR		SGVFNSTFYGFYFNRLQGE	20.0	20.5	21.6	1.0
H2CB-4-E5-IGFR		GYREMSDLGFYGFYFNRLQGL	21.6	20.5	21.2	1.0
H2CB-4-E8-IGFR		SVFMQHDHGFYGFYFNRLQMBE	22.0	19.9	20.9	1.0
H2CB-3-D12-IGFR		FRHITVDVRSFYGFYFNRLQAG	21.1	19.7	20.7	1.0
H2CB-4-G9-IGFR		WAGSDVDSFYGFYFNRLQAG	26.6	17.3	16.8	1.0
H2CB-3-C8-IGFR		GLQVNSFHSGFYGFYFNRLQVQ	21.6	14.5	15.2	1.0
H2CB-3-A12-IGFR		SRVSDFYHGFYGFYFNRLQVQ	20.8	13.4	13.9	1.0
H2CB-3-B12-IGFR		MGATFTHTGFYGFYFNRLQVQ	28.6	27.5	29.2	0.9
H2CB-3-A9-IGFR		RPASRPFHSGFYGFYFNRLQVQ	27.8	25.2	27.1	0.9
H2CB-3-A3-IGFR		GLAFGNFTHDFYGFYFNRLQVQ	27.7	24.3	25.7	0.9
H2CB-3-B4-IGFR		TAAISDENSIFYGFYFNRLQVQ	26.9	24.1	26.5	0.9
H2CB-4-E7-IGFR		LDELLPQAGFYGFYFNRLQVQ	23.8	23.8	25.3	0.9
H2CB-4-G6-IGFR		ASHKSAPDNDFYGFYFNRLQVQ	24.6	21.6	24.0	0.9
H2CB-4-E9-IGFR		HTGAGDLHGAFYFNRLQVQ	22.4	21.1	23.0	0.9
H2CB-4-H2-IGFR		RRGRDGFHGFYGFYFNRLQVQ	24.3	20.7	22.0	0.9
H2CB-3-A10-IGFR		GNFREAFAHGFYGFYFNRLQVQ	24.3	20.2	21.9	0.9
H2CB-3-C4-IGFR		RDLPAPAFQAFYGFYFNRLQVQ	21.6	19.9	21.5	0.9
H2CB-3-B5-IGFR		ERETAFAQAFYGFYFNRLQVQ	23.1	19.2	22.0	0.9
H2CB-4-G4-IGFR		WGEGGCFYDWFYGFYFNRLQVQ	24.2	18.7	20.7	0.9
H2CB-3-D9-IGFR		SLVAADLHGFYGFYFNRLQVQ	21.7	18.7	21.2	0.9
H2CB-3-C3-IGFR		TSEVGDFAHGFYGFYFNRLQVQ	24.4	18.6	20.0	0.9
H2CB-3-D3-IGFR		TGADGLLHGFYGFYFNRLQVQ	20.3	18.4	21.1	0.9
H2CB-4-F2-IGFR		RRSDSLHRSFYGFYFNRLQVQ	22.5	18.3	21.3	0.9
		SESKYLLHSFYGFYFNRLQVQ	18.0	16.8	18.3	0.9

Figure 1N (Con't)

Clone	Sequence	Ratios over Background		Comparisons	
Design		E-Tsg	IR	IGFR/IR	IR/IGFR
H2CB-4-H1-IGFR	XXXXXXXXXXFYWFXXXXXX	--	--	--	--
H2CB-4-F9-IGFR	HGVIRADITGFGWFSQQLSD	18.3	15.3	16.5	0.9
H2CB-4-E10-IGFR	LINA.VFRGFYAMFEQVSK	22.9	14.4	15.3	0.9
H2CB-4-F8-IGFR	LQRYIGFHDFFYDNFSALSG	26.1	20.1	24.5	0.8
H2CB-3-A8-IGFR	MRTAELFHVGFGYDNFDAQLM	21.5	14.8	19.0	0.8
H2CB-4-F1-IGFR	WAPPDALHGQFYRWFQQLDQ	20.7	14.7	18.2	0.8
H2CB-3-C6-IGFR	AVHAATFHDDFYRWFQVWGS	22.2	14.6	18.8	0.8
H2CB-4-E11-IGFR	PDAVHGDFGFGYWFKEQLOR	15.7	7.8	10.2	0.8
H2CB-3-D6-IGFR	QAGGMEFHGAFYWFELQLSG	26.1	17.6	24.1	0.7
H2CB-4-F3-IGFR	GRSGRMNAEFGWFGHQLAA	21.6	13.0	18.8	0.7
H2CB-3-A4-IGFR	AAVNSLPHDEFYLFQDQLDG	17.3	11.1	16.4	0.7
H2CB-3-B1-IGFR	QLGMWFHADFYWFELQPLPS	27.4	11.0	14.8	0.7
H2CB-3-C5-IGFR	RLAGSGIHGFGYWFVQQLLA	20.0	11.0	15.2	0.7
H2CB-4-F6-IGFR	GREIGGVHDGFGYWFQCSQ	19.9	10.5	15.6	0.7
H2CB-3-B8-IGFR	VRSEQRFDSSFGYWFNDLLMS	18.6	10.1	14.6	0.7
H2CB-3-C7-IGFR	QSPYGFPHDGFYWFLOQTOM	20.7	6.9	9.5	0.7
H2CB-4-H7-IGFR	FQCGAAPHVDYRWFTEQOBF	16.2	1.8	2.5	0.7
H2CB-4-F5-IGFR	GAFGSEPHQFYRWFEDALSF	21.8	14.1	22.7	0.6
H2CB-4-G1-IGFR	EHTSYQIHROFYWFDALGR	12.9	4.0	7.2	0.6
H2CB-3-D11-IGFR	SGTAADLHSRFGYWFALQARE	20.4	10.3	19.7	0.5
H2CB-3-D7-IGFR	EGFGVLFHGQFYRWFQQLDG	24.1	8.8	18.6	0.5
H2CB-3-C10-IGFR	QQSAGPHSSFGYWFSELLGA	22.1	6.5	13.6	0.5
H2CB-4-E3-IGFR	YLQAGFHRSGFYWFDQLRSD	21.7	5.1	10.4	0.5
H2CB-3-C1-IGFR	MLWATLHSDFYWFEQVWSG	20.3	4.6	8.9	0.5
H2CB-4-G2-IGFR	GANALGFKDRFYWFEQAQLMD	22.3	6.7	15.7	0.4
H2CB-3-A11-IGFR	GSGLYVFWHMGFYWFEQMGGS	19.9	3.3	10.7	0.3
H2CB-4-F5-IGFR	LDKMGFGDLQFYRWFARATRA	23.9	2.5	7.7	0.3
H2CB-4-G12-IGFR	QRSAVEFHADFYWFDLRLTLP	19.3	2.5	7.9	0.3
H2CB-4-F12-IGFR	DQRMGSFHGFGFYRWFETLLS	16.7	1.7	5.4	0.3

Figure 1N (Con't)

Clone Design	Sequence $X_n - Fy \times WF - X_n$	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IGFR/IR	IR/IGFR
20E2A-3-B11-IR	GRFYGMFQDAIDOLMPWGFDP	24.6	1.4	23.6	0.1
20E2B-3-E3-IR	IQGMEFFYGNFDDVVAQMPDE	23.0	0.9	15.3	0.1
1B6-3-F6-IR	RYGRWGIAQFYDMFDR	40.9	1.0	13.3	0.1
1B6-4-F9-IR	RRLGSLSTQFYMFAR	34.1	1.0	12.6	0.1
20E2B-3-A8-IR	ASATPFYQMFADVSEYMQQ	35.4	7.4	34.4	0.2
A6L-4-F6-IR	PYRMGTEKNFDMFVQLQ	28.9	4.1	18.1	0.2
20E2B-4-H9-IR	SAVHFQYKMFNDLLEPLSA	37.8	9.4	26.7	0.4
20E2B-3-B1-IR	VPVKSFYRMFQVLGSSDW	41.8	12.9	36.8	0.4
20E2B-4-F9-IR	QSPRASYGNFDDVLRAGVV	25.9	4.2	10.1	0.4
20E2B-3-E9-IR	TGFYEMFYQLHSNLPFLD	27.0	7.7	17.2	0.5
20E2B-3-E10-IR	RRVGGFYGFNSQQLQMGVA	22.2	2.6	5.5	0.5
20E2B-3-C12-IR	SSQDRRFYMFQALVGGHDG	39.0	6.7	12.0	0.6
20E2B-3-E7-IR	TRGQLGFYMFQALSTGSG	20.2	2.2	3.8	0.6
20E2B-3-E11-IR	CADLNAPYQMFQGLDRGSDH	9.2	1.2	1.9	0.6
20E2B-3-B11-IR	TLIQDQFYMFSDLLSAEFGD	20.7	1.3	2.1	0.6
20E2B-4-G2-IR	IDQLDAFYMFQGLMGDP	36.0	20.7	32.8	0.6
20E2B-3-A7-IR	RGGLTFYMFESALRKHGAG	10.8	6.3	8.9	0.7
20E2B-4-C12-IR	RGLQDQFYMFQNLVGYEYDR	19.0	4.2	5.5	0.8
20E2B-3-C11-IR	MQGHRGYGMFARVLEQDRGM	37.0	22.3	29.5	0.8
20E2B-3-C10-IR	ERLHLRFYMFQVITVGGQSD	37.3	26.8	34.8	0.8
	MHVQSDFYHMFQSLIGQGQPD	37.7	24.8	30.5	0.8

Figure 10

Clone Design	Sequence $X_n - Fy\alpha F - X_n$	Ratios over Background		Comparisons	
		E-Tag	IGFR	IGFR/IR	IR/IGFR
20E2Ba-3-D7-IR	TWGTGFRYFNQVUKEHLSG	35.4	26.9	31.3	0.9
20E2Ba-3-A12-IR	ITHNRGYSNFDLVVQGGAGA	31.7	22.0	23.3	0.9
20E2Ba-3-D10-IR	VRDAGFYQWFADILTOLDFE	32.7	27.3	29.1	0.9
20E2Ba-4-G7-IR	MOLQDEFYNFRGIMLNDQOD	34.2	29.0	30.7	0.9
20E2Ba-4-F5-IR	GIRSGFYQWFDRLVAGVGDG	33.8	32.1	34.0	0.9
20E2Ba-3-C9-IR	ANLNSQFYSWFSVITGEASFS	39.4	33.2	35.5	0.9
20E2Ba-3-A4-IR	QSPFRASFYGMFDDVLRAAGVV	38.2	31.6	35.9	0.9
20E2Ba-4-E12-IR	MORNOGYSNFDDLVSVTVGV	36.0	30.8	29.7	1.0
20E2Ba-4-E11-IR	ASGDFPYANFLEQLRVANGS	35.1	31.2	30.7	1.0
20E2Ba-4-E8-IR	SGTPYGFYRWFQSLASATSG	36.1	30.5	30.7	1.0
20E2Ba-4-H10-IR	QVGGGTYEWFDRAMGDVRPW	38.9	30.6	30.7	1.0
20E2Ba-4-F6-IR	DNMSGGFYRWFQVAVDSGGD	34.9	33.2	32.0	1.0
20E2Ba-4-G4-IR	RGTDITFYGMFDQLQGWCCD	34.1	33.7	32.2	1.0
20E2Ba-4-F8-IR	TVDTHTQFYDMFSRVLGSGSA	37.7	32.0	32.7	1.0
20E2Ba-4-G5-IR	GRQDREFYWFELQAGMGDD	34.9	33.9	33.4	1.0
20E2Ba-3-B10-IR	RILLGGFYENFDQVLKTEKV	38.2	34.9	33.6	1.0
20E2Ba-3-C7-IR	GVLTSGFYENFALQLHLGLAG	37.6	34.2	34.8	1.0
20E2Ba-3-C5-IR	PAVGQSFYGMFEAVLRSGKAG	40.4	36.0	35.6	1.0
20E2Ba-3-B9-IR	SNGISGFYEMFAAQVOTSDFO	39.6	35.8	37.1	1.0
A6L-4-F11-IR	LLGLSQAAYANFYDMFVSQILA	33.1	4.6	4.6	1.0
20E2Ba-3-C2-IR	VPNMSWTFYNFAFQIEGSEGE	44.1	40.0	38.1	1.0
20E2Ba-3-B2-IR	AREADGFYDMFROVSGSAVO	43.1	40.1	39.0	1.0
20E2Ba-4-G2-IR	GVVEGTFYEMFDRLGGVQGD	34.1	33.6	29.8	1.1
20E2Ba-4-H6-IR	SHLTDPFYQWFDQLRAGVRG	39.4	36.0	31.9	1.1

Figure 10 (Con't)

Clone	Design	Sequence X_n -Fy α F- X_n	E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
20E2B α -4-H5-IR		RSNDADFYRWFNIIQVGGG	38.7	35.1	32.3	1.1	0.9
20E2B α -4-G3-IR		DSGDAFYIWFEDQLRSAGWD	35.5	36.1	32.7	1.1	0.9
20E2B α -4-H4-IR		PGLHRAFYQWPAEVRANKKE	38.8	37.9	35.0	1.1	0.9
20E2B α -4-C1-IR		SLGGGGFYDMFASQVGGADI	43.7	42.1	39.0	1.1	0.9
20E2B α -4-E6-IR		CGQTSFYQWCFEVARVESGD	38.0	34.3	29.7	1.2	0.9
H5-3-D5-IR		IWVEGTQGVNFYDMFVKQLQ	43.6	21.8	18.2	1.2	0.8
JBA5-3-D9-IR		RUVMSGSASTNFYDMFVQLG	38.3	29.8	25.3	1.2	0.8
20E2B β -4-G6-IR		SGAGSAFYMFQVILRTVHSA	22.4	6.2	1.9	3.3	0.3
20E2B β -4-H10-IR		SNGLSGFTMFPAQVQVTSDFQ	23.5	32.2	9.7	3.3	0.3
xB6-4-G8-IR		RDRRGGLDVFFYQMFMD	--	--	--	--	--

Figure 10 (Con't)

000250-05000000

Clone Design	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
R40-3-40H4-IR	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX	--	--	--	--	--
	RYFPFGFYGNLDVLRWLRPYVASPRNGHWRPGSLGKQPT	31.9	1.4	2.1	0.7	1.5

FIGURE 2A

Clone	Sequence	Ratios over Background		Comparisons	
Design		E-Tag	IR	IGFR/IR	IR/IGFR
R40-3-B6-IGFR	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX	--	--	--	--
R40-X-E5-IGFR	AETPAQVGNLWSVPGEHNTVDPFYHKLSELLRESGA	--	--	--	--
R40-X-B5-IGFR	RHLTNAELGVQSPVLRSRLFPDGDIFYRALSHLRGMGPP	--	--	--	--
R40-4-9-IGFR	RGMDRQWLDVGARHLERRSVQNTDDFYGGLRILVDGF	--	--	--	--
R40-3-G6-IGFR	GPDSFDVTEKGMALINVRFDPSLDFNDQTFYFLDLSL	--	--	--	--
R40-4-12-IGFR	GGTYFRGQVAGSNESILRVNFIQLLEALASPRT	--	--	--	--
R40-3-A5-IGFR	APFDARLSAPRFQWSPRTWXQSLSYGENSCSFYDCLSI	--	--	--	--
R40-X-C6-IGFR	MGSSQFDITRESSQAYSHSLSDSDGMGTANWIFRLAEGGL	--	--	--	--
	SGAAHEGNQGRERSTHLAANINDHLPDGDGILWLGYSWLS	--	--	--	--

FIGURE 2B

Clone Design	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR	--
R20 α -4-20C11-IR	XXXXXXXXXXXXXXXXXXXXX	43.7	30.8	3.0	10.3	0.1	
R20 α -3-20E2-IR	DRAFYNGRLDLVGAVYGWD	46.3	39.9	3.1	12.9	0.1	
R20 α -4-20A12-IR	FYDAIDQLVRGSARAGGTRD	48.6	39.9	2.4	16.6	0.1	
R20 β -4-C6-IR	RLFYCGIQALGANLGYSGCV	18.5	28.9	4.3	6.7	0.1	
R20 β -4-A6-IR	FYSALWGLCGVTGCG	9.3	25.9	1.5	17.3	0.1	
	RQSDAFYSGLWALIGLSDG						

FIGURE 2C

Clone	Design	Sequence	Ratios over Background		Comparisons	
			E-Tag	IR	IGF1R	IR/IGF1R
		XXXXXXXXXXXXXXXXXX	--	--	--	--
R20-4-F11-IGFR		GFYELGALVGERVGTNS	39.0	19.2	--	--
R20-4-C7-IGFR		ERTDPFYKALLSLGGDSGS	33.4	17.5	--	--
R20-3-F2-IGFR		DVQNGSGFYDGIIFGLAWG	31.8	14.3	--	--
R20-4-A11-IGFR		PFYWIIRDLGPPLPHTRGD	37.8	13.5	--	--
R20-4-B12-IGFR		VLVVGGLDPFYEGHLRLIS	37.2	10.0	--	--
R20-4-B10-IGFR		GFYRLNLELVREGGALKVGA	37.0	9.5	--	--
R20-4-E9-IGFR		GQRGFYELLSELLGHEGGVF	34.2	9.4	--	--
R20-3-H4-IGFR		DWVSGPFYRGTELLSGFQIE	30.3	7.8	--	--
R20-3-G2-IGFR		GGSLFYEGGLRLVLGDSVVG	20.8	6.9	--	--
R20-4-B8-IGFR		LNHFYAMLSDLGSVRNIFPG	32.8	6.5	--	--
R20-4-E7-IGFR		LSGFYEGFLRLARRDGSWG	35.4	6.4	--	--
R20-4-G9-IGFR		FYDVLGALVGVLEGEQCDAS	25.0	6.4	--	--
R20-4-D9-IGFR		GAGSFGREGGFYALMLAG	23.4	6.3	--	--
R20-4-D11-IGFR		DDEFYSQLLVGDSRGSGTQN	31.3	4.0	--	--
R20-4-G10-IGFR		PFYMLLSRLVGVEQEGGL	13.6	3.3	--	--
R20-4-C8-IGFR		FYDAIDQLVRGSANAGSTRD	16.8	3.2	--	--

FIGURE 2D

Clone	Parental/Design	Sequence	Ratios over Background		Comparisons	
			E-Tag	IR	IGFR/IR	IR/IGFR
B6L-4-C8-IR		AETPAQVGNRLSVMPGEHNTVDPFYHKLSELLRESGA	40.5	6.1	40.8	0.2
B6L-4-C8-IR		ANILRIRVGNRLSVMPGEHNTVDPFYHKLSELLRESGA	19.6	4.0	23.5	0.2
B6L-4-B7-IR		AETPAQVGNRLSVMPGEHNTVDPFYHKLSELLRESGD	20.6	2.8	2.7	0.9
B6L-3-H1-IR		AETPAHVC*TVGGLFGRVNTVNTVDPFYHKLSELLRESGA	15.5	4.1	3.0	1.1
B6L-4-E12-IR		GQNGSAMDGIGLSVMPGDVNPVDPFYHKLSELLRESGA	36.0	9.4	8.5	1.1
B6L-4-D8-IR		AETPAQVGNRLSVMPGEHNTVDPFYHKLSELLRESGA	37.8	24.6	20.6	1.2
B6L-4-F7-IR		AETPAQVGNRLSVMPGEHNTVDPFYHKLSELLRESGD	5.5	2.0	1.6	1.3
B6L-4-B11-IR		AETPAQVGNRLSVMPGEHNTVDPFYHKLSELLRESGA	6.8	2.0	1.6	1.3
B6L-4-B12-IR		AETPAQVGNRLSVMPGEHNTVDPFYHKLSELLRESGA	36.4	18.7	14.2	1.3
B6L-4-B8-IR		T*QGETPAQVSVMPGEHNTVDPFYHKLSELLRESGA	35.6	11.4	8.6	1.3
B6L-4-E8-IR		QGETPAQVGNRLSVMPGEHNTVDPFYHKLSELLRESGA	7.6	2.5	1.8	1.4
B6L-3-G6-IR		VDTPAQVGNRLSVMPGEHNTVDPFYHKLSELLRESGA	11.5	2.0	1.4	1.4
B6L-3-G5-IR		AETSAQVGNRLSVMPGEHNTVDPFYHKLSELLRESGA	14.8	3.2	2.2	1.5
B6L-4-E10-IR		*NSPRVGNRLSVMPGEHNTVDPFYHKLSELLRESGV	26.2	11.5	7.2	1.6
B6L-4-F10-IR		AETPAQVGNRLSVMPGEHNTVDPFYHKLSELLRESGP	36.0	17.1	10.1	1.7
B6L-4-A7-IR		ADTPAQVGNRLSVMPGEHNTVDPFYHKLSELLRESGA	11.6	3.4	1.9	1.8
B6L-4-A7-IR		AGTPAQV*GNRLSVMPGEHNTVDPFYHKLSELLRESGA	30.4	11.2	5.9	2.0
B6L-4-G8-IR		D*QAMVMPQGHNTIDPFYHKLSELLRESGA	35.6	12.8	7.2	2.0
B6L-4-F8-IR		AETLRVGNRLSVMPGEHNTVDPFYHKLSELLRESGS	33.5	12.9	6.4	2.3
B6L-4-G7-IR		AATRFQVGNRLSVMPGEHNTVDPFYHKLSELLRESGS	16.9	6.3	2.7	2.4
B6L-3-F4-IR		LTTPAQVGNRLSVMPGEHNTVDPFYHKLSELLRESGA	20.6	4.9	2.0	2.5
B6L-3-H4-IR		ADNPQVGNRLSVMPGEHNTVDPFYHKLSELLRESGA	22.4	6.3	3.2	3.2
B6L-3-A6-IR		AETPAQVGNRLSVMPGEHNTVDPFYHKLSELLRESGA	14.3	4.8	1.4	3.4
B6L-4-D7-IR		AETSVQVGNRLSVMPGEHNTVDPFYHKLSELLRESGA	29.2	16.7	3.8	6.3
B6L-3-E2-IR		G*NSAIVGNRLSVMPGEHNTVDPFYHKLSELLRESGV				

FIGURE 2F

Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IR	IGFR/IR	IR/IGFR
B6L-4-G6-1GFR	AETPAQVGNRLWSVMPGEHNTVDPFYHKLSELLRESGA	7.3	--	--	--
B6L-4-G10-IGFR	AETPAQVGGDRLWSVMPGEHNTVDPFYHKLSELLRESGA	22.1	--	--	--
B6L-4-G10-IGFR	AE?PAQVGNRLWSVMPGEHNTVDPFYHKLSELLRESGA	5.5	--	--	--
B6L-4-G3-IGFR	AETPAQVGNRLWSVMPGEHNTVDPFYHKLSELLRESGA	5.8	--	--	--
B6L-3-F10-IGFR	AETPAQVGNRLWSVMPGEHNTVDPFYHKLSELLRESGA	6.7	--	--	--
B6L-4-D2-IGFR	AETPAQVGNRLWSVMPGEHNTVDPFYHKLSELLRESGA	6.5	--	--	--
B6L-3-H10-IGFR	AETPAQVGNRLWSVMPGEHNTVDPFYHKLSELLRESGA	15.9	--	--	--
B6L-4-B12-IGFR	AETPAQVGNRLWSVMPGEHNTVDPFYHKLSELLRESGA	5.9	--	--	--
B6L-3-A9-IGFR	DETSAQVGNRLWSVMPGEHNTVDPFYHKLSELLRESGA	6.3	--	--	--
B6L-4-C4-IGFR	GETPAQVGNRLWSVMPGEHNTVDPFYHKLSELLRESGA	14.8	--	--	--
B6L-4-E3-IGFR	AETPAQVGNRLWSVMPGEHNTVDPFYHKLSELLRESGA	5.2	--	--	--
B6L-4-A12-IGFR	AETPAQVGNRLWSVMPGEHNTVDPFYHKLSELLRESGA	5.4	--	--	--
B6L-4-D5-IGFR	AEAPDQVGNRLWSVMPGEHNTVDPFYHKLSELLRESGA	11.1	--	--	--
B6L-3-B9-IGFR	PETPAQVGNRLWSVMPGEHNTVDPFYHKLSELLRESGA	3.0	--	--	--
B6L-3-A10-IGFR	AQTPAQVGNRLWSVMPGEHNTVDPFYHKLSELLRESGA	4.7	--	--	--
B6L-3-H9-IGFR	AETPAQVGNRLWSVMPGEHNTVDPFYHKLSELLRESGA	10.9	--	--	--
B6L-4-A5-IGFR	AETPAQVGNRLWSVMPGEHNTVDPFYHKLSELLRESGA	9.1	--	--	--
B6L-3-G10-IGFR	AETPAQVGNRLWSVMPGEHNTVDPFYHKLSELLRESGA	2.9	--	--	--
B6L-4-F11-IGFR	AETPAQVGNRLWSVMPGEHNTVDPFYHKLSELLRESGA	4.5	--	--	--
B6L-3-G11-IGFR	AETPAQVGNRLWSVMPGEHNTVDPFYHKLSELLRESGA	8.0	--	--	--
B6L-4-D4-IGFR	AETPAQVGNRLWSVMPGEHNTVDPFYHKLSELLRESGA	2.4	--	--	--
B6L-4-F12-IGFR	AETPAQVGNRLWSVMPGEHNTVDPFYHKLSELLRESGA	7.5	--	--	--
B6L-4-E12-IGFR	AETPAQVGNRLWSVMPGEHNTVDPFYHKLSELLRESGA	4.2	--	--	--
B6L-4-E10-IGFR	AETPAQVGNRLWSVMPGEHNTVDPFYHKLSELLRESGA	2.5	--	--	--
B6L-3-G9-IGFR	AETPAQVGNRLWSVMPGEHNTVDPFYHKLSELLRESGA	6.8	--	--	--
B6L-4-E11-IGFR	DETPAHVGNRLWSVMPGEHNTVDPFYHKLSELLRESGA	2.1	--	--	--
B6L-4-E12-IGFR	ACTPAQVGNRLWSVMPGEHNTVDPFYHKLSELLRESGA	6.4	--	--	--
B6L-4-E12-IGFR	AETPAQVGNRLWSVMPGEHNTVDPFYHKLSELLRESGA	2.7	--	--	--
B6L-4-E10-IGFR	AETPAQVGNRLWSVMPGEHNTVDPFYHKLSELLRESGA	3.9	--	--	--
B6L-3-G9-IGFR	AETPAQVGNRLWSVMPGEHNTVDPFYHKLSELLRESGA	1.9	--	--	--
B6L-3-G9-IGFR	AETPAQVGNRLWSVMPGEHNTVDPFYHKLSELLRESGA	1.8	--	--	--
B6L-3-G9-IGFR	AETPAQVGNRLWSVMPGEHNTVDPFYHKLSELLRESGA	3.6	--	--	--
B6L-3-G9-IGFR	AETPAQVGNRLWSVMPGEHNTVDPFYHKLSELLRESGA	1.2	--	--	--
B6L-3-G9-IGFR	AETPAQVGNRLWSVMPGEHNTVDPFYHKLSELLRESGA	2.5	--	--	--

FIGURE 2G

Clone Design	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGFSR	IR	IGFSR	IR	IGFSR
B6Ha-3-F5-IR	OOUUUUUUJJJDPFYHKLSELXXOO	42.7	9.6	17.9	0.5	1.9	1.9
B6Ha-2-D10-IR	GGAVAAVGSRADPFYHKLSELVQGS	20.8	1.7	1.1	1.5	0.6	0.6
B6Ha-3-F1-IR	SGGQQRKAATSDDPFYHKLSELLGG	22.5	2.4	1.3	1.8	0.5	0.5
B6Ha-3-E6-IR	CSMAAVERAGDDDPFYHKLSELQGS	18.2	2.3	1.2	1.9	0.5	0.5
B6Ha-1-B8-IR	CGAKMTGTFNDFYHKLSELLQRG	44.6	5.2	2.1	2.5	0.4	0.4
B6Ha-2-D5-IR	CCVEAEAVGRRGDPFYHKLSELTGCC	39.6	2.3	0.9	2.6	0.4	0.4
B6Ha-1-B3-IR	SRVTWVIKRGSDPFYHKLSELVQGR	33.1	3.2	1.1	2.9	0.3	0.3
B6Ha-3-E5-IR	GCITAEANGAGDPFYHKLSELGCS	28.8	2.9	1.0	2.9	0.3	0.3
B6Ha-4-H9-IR	RCGDEBQENRRDDPFYHKLSELFGCC	17.4	6.4	2.1	3.0	0.3	0.3
B6Ha-2-D8-IR	GCEVIAAEGRRDDPFYHKLSELQCGG	19.3	3.0	1.0	3.0	0.3	0.3
B6Ha-3-E4-IR	SSETAKMTGTTRDDPFYHKLSELVQGS	43.1	8.7	2.8	3.1	0.3	0.3
B6Ha-3-F7-IR	WLCGGWQKRRPGDPFYHKLSELIDCG	41.5	3.1	1.0	3.1	0.3	0.3
B6Ha-1-A3-IR	SRVAATKEKRPSDDPFYHKLSELQGS	37.4	2.6	0.8	3.3	0.3	0.3
B6Ha-4-H10-IR	SRAKVEAMPDSGDPFYHKLSELLASG	50.5	29.5	8.6	3.4	0.3	0.3
B6Ha-3-F3-IR	GGAAKTTVVGSDPFYHKLSELQGS	48.9	19.7	5.7	3.5	0.3	0.3
B6Ha-3-F6-IR	CGVGEQMEVTDGDDPFYHKLSELLWSC	18.1	15.6	4.3	3.6	0.3	0.3
B6Ha-4-G8-IR	SGEQATIEGPNOPFYHKLSELIWGS	32.3	6.1	1.7	3.6	0.3	0.3
B6Ha-2-D1-IR	GGTKAVAKVTGTRDDPFYHKLSELQGS	11.7	5.4	1.3	4.2	0.2	0.2
B6Ha-2-D6-IR	GCEVIEEGDSADPFYHKLSELQGS	47.0	5.6	1.3	4.3	0.2	0.2
B6Ha-3-F10-IR	GCAVVEAERSRGDPFYHKLSELIOGC	33.5	4.4	1.0	4.4	0.2	0.2
B6Ha-3-E8-IR	GRTMAVMAAGPDDPFYHKLSELVQGG	47.2	8.8	1.9	4.6	0.2	0.2
B6Ha-2-C10-IR	GCVEWQKHGASDPFYHKLSELGCS	47.6	5.3	1.1	4.8	0.2	0.2
B6Ha-2-C7-IR	RGKTAIVVTGSDPDPFYHKLSELVQGS	46.9	5.8	1.1	5.3	0.2	0.2
	SGAKVIVVTGSDPDPFYHKLSELVQGS	45.1	6.7	1.0	6.7	0.1	0.1
	RGIVAMVEATEVSGSDHPFYHKLSELVQGS						

FIGURE 2H

Clone Design	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGF5R	IR	IGF5R/IR	IR/IGF5R	IR/IGF5R
B6H0-1-A6-IR	OOUUUUUUJJDDPFYHKLSELX00	--	--	--	--	--	--
B6H0-2-C5-IR	GCKMETETGSDDPFYHKLSELCSGG	49.7	34.5	4.0	8.6	0.1	0.1
B6H0-2-C4-IR	RGEVATMEVPAGDDPFYHKLSELLWGS	42.6	34.2	3.3	10.4	0.1	0.1
B6H0-2-C9-IR	RCGRW*AEWGAGDDPFYHKLSELVCG	20.7	9.9	0.9	11.0	0.1	0.1
B6H0-4-H8-IR	RCMVETIAGVGDDPFYHKLSELQGG	47.4	32.6	2.8	11.6	0.1	0.1
B6H0-3-F11-IR	WWOKKSGDGASDDPFYHKLSELLWGS	36.3	28.1	2.4	11.7	0.1	0.1
B6H0-3-E9-IR	RCMEKVLVGGSDPFYHKLSELLQGS	49.5	18.7	1.6	11.7	0.1	0.1
B6H0-1-B5-IR	RCEEKQAEVGFSDPFYHKLSELLGCR	44.6	24.2	1.7	14.2	0.1	0.1
B6H0-1-A2-IR	RGCNDDGKGNSDDPFYHKLSELLCGG	22.3	14.6	1.0	14.6	0.1	0.1
B6H0-3-G4-IR	CCTTEWVMWDARDDPFYHKLSELVTGG	41.5	20.5	1.0	20.5	0.0	0.0
B6H0-3-D9-IR	GCKKVEAKKGNDADPFYHKLSELLQGC	36.4	28.4	36.0	0.8	1.3	1.3
B6H0-3-A10-IR	RSMMAKAIVGGGDDPFYHKLSELQFSR	36.7	27.9	34.7	0.8	1.2	1.2
B6H0-3-A3-IR	CGGAVPDGDDPFYHKLSELLMQCC	34.9	32.1	35.6	0.9	1.1	1.1
B6H0-3-G1-IR	GCCEEVETTGHRDDPFYHKLSELLQGC	36.3	33.7	37.3	0.9	1.1	1.1
B6H0-3-B3-IR	GCAEIEIAAGGGDDPFYHKLSELLQGC	34.7	33.7	35.9	0.9	1.1	1.1
B6H0-3-F5-IR	GCAEVKAVKGAGDDPFYHKLSELLQGC	35.9	35.1	37.4	0.9	1.1	1.1
B6H0-3-G11-IR	GCAAVETTINGNDPFYHKLSELLQGC	37.6	36.2	39.0	0.9	1.1	1.1
B6H0-3-F3-IR	CGEVTGRAGDDPFYHKLSELLQGC	39.2	37.2	41.0	0.9	1.1	1.1
B6H0-3-A1-IR	GCMVVEATGRRHDDPFYHKLSELLQGC	41.3	38.0	43.0	0.9	1.1	1.1
B6H0-3-H3-IR	GCTEVVGGSDDPFYHKLSELLQGC	39.0	38.3	40.7	0.9	1.1	1.1
B6H0-3-D3-IR	QCAMEEIRGANDPFYHKLSELLCEGG	38.8	38.4	41.3	0.9	1.1	1.1
B6H0-3-C9-IR	GCAELIVIEGDDSDPFYHKLSELLQGC	36.7	39.2	41.5	0.9	1.1	1.1
B6H0-3-F1-IR	PQCSSIRAEKSGDDPFYHKLSELLVGC	41.5	40.0	42.2	0.9	1.1	1.1
B6H0-3-C6-IR	GCAAVVAEASGDDPFYHKLSELLQGC	39.9	40.3	42.7	0.9	1.1	1.1

FIGURE 2H (Con't)

Clone Design	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGFSR	IR	IGFSR/IR	IR/IGFSR	
B6H β -3-D7-IR	OOUUUUUUUUJJDDPFYHKLSELXXOO	39.5	27.1	26.3	1.0	1.0	1.0
B6H β -3-B2-IR	RGVEMKAAIUWGTENDPFYHKLSELSSGS	34.3	34.4	35.4	1.0	1.0	1.0
B6H β -3-G5-IR	CSAVKMAEAGDPSDPFYHKLSELQGS	35.3	35.0	35.6	1.0	1.0	1.0
B6H β -3-H1-IR	RGDGDPPFYHKLSELQGS	36.8	35.4	36.5	1.0	1.0	1.0
B6H β -3-A5-IR	WLCKRQTHDPPFYHKLSELACGR	34.9	35.5	35.9	1.0	1.0	1.0
B6H β -3-H11-IR	SSKVRKATVGTDPDPFYHKLSELQGS	37.7	36.4	37.6	1.0	1.0	1.0
B6H β -3-C2-IR	CCAAIAVATGNDNDPFYHKLSELQGR	37.4	36.5	37.2	1.0	1.0	1.0
B6H β -3-C6-IR	GCAAIVKETHDPPDPFYHKLSELHGC	37.0	37.7	39.5	1.0	1.0	1.0
B6H β -3-A11-IR	SCAAEKEVAGTARDPFYHKLSELQGS	40.4	38.2	39.1	1.0	1.0	1.0
B6H β -3-D8-IR	CSVAVGSDGDPFYHKLSELQGR	35.4	38.3	39.5	1.0	1.0	1.0
B6H β -3-B7-IR	WQRNKQIIGTDPDPFYHKLSELQGS	37.8	39.0	39.4	1.0	1.0	1.0
B6H β -3-A12-IR	RSAAAIVGSPNDPFYHKLSELQGS	33.5	39.4	41.3	1.0	1.0	1.0
B6H β -3-B4-IR	WLCIDRDGRDEQWDPFYHKLSELVSGR	39.0	39.8	41.1	1.0	1.0	1.0
B6H β -3-A4-IR	GSVAAAAKTSGSDDPFYHKLSELQGS	40.1	40.4	41.1	1.0	1.0	1.0
B6H β -3-E12-IR	GCAVTTWTRSPADPFYHKLSELQGR	35.8	40.7	40.7	1.0	1.0	1.0
B6H β -3-B8-IR	CKKVDD*ARSSDPFYHKLSELQGR	40.8	40.7	39.5	1.0	1.0	1.0
B6H β -3-C5-IR	CKKAVVEVKGDDPFYHKLSELQGC	40.7	40.9	42.6	1.0	1.0	1.0
B6H β -3-A2-IR	CSTVTVSGSDDPFYHKLSELQGC	41.1	41.4	41.9	1.0	1.0	1.0
B6H β -3-A8-IR	RSVTAKVEVGSDPFYHKLSELQGS	40.0	41.9	41.6	1.0	1.0	1.0
B6H β -3-B11-IR	GSRQKIEVGTDPDPFYHKLSELQGG	39.8	42.0	41.3	1.0	1.0	1.0
B6H β -3-C12-IR	LCDEKQRTVGTNDPFYHKLSELTGCR	40.7	42.6	43.3	1.0	1.0	1.0
B6H β -3-B3-IR	SCWVEGNDDPFYHKLSELQGR	43.0	42.7	44.0	1.0	1.0	1.0
B6H β -3-B5-IR	GGAAVVVWVGNDPFYHKLSELQGG	42.7	43.9	45.4	1.0	1.0	1.0
B6H β -3-C4-IR	GGVIKAMKAGGDDPFYHKLSELQGS	41.1	42.3	36.6	1.2	0.9	0.9
B6H β -3-G7-IR	CCCIKAVVGGDDPFYHKLSELQGC	4.1	2.4	2.1	1.1	0.9	0.9
B6H β -3-C11-IR	CCERKAVVAGNDPFYHKLSELQGC	29.2	30.6	28.2	1.1	0.9	0.9

FIGURE 2H (Con't)

Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IR	ICFR/IR	IR/ICFR
B6H β -3-H12-IR	OOUUUUUUJLLJDPFYHKLSELXXOO	--	--	--	--
B6H β -3-G10-IR	RGEAKIGSAGDPFYHKLSELMOGR	33.6	32.0	29.5	1.1
B6H β -3-F10-IR	GCEVVMWANSADPFYHKLSELQGR	30.1	34.3	30.5	1.1
B6H β -3-D5-IR	GCAVVTGGNDPFYHKLSELQGR	37.1	35.3	32.4	1.1
B6H β -3-B12-IR	SRTGERQVGVSHADPFYHKLSELSS	39.9	38.9	35.5	1.1
B6H β -3-D2-IR	GCKEVVETAHDDPFYHKLSELQGR	39.5	40.0	37.1	1.1
B6H β -3-D1-IR	RRITIKVAGDDDDPFYHKLSELMOG	40.4	41.5	39.1	1.1
B6H β -3-G6-IR	WCDQKETVNSDDPFYHKLSELVGS	41.1	44.6	36.6	1.2
B6H β -3-A7-IR	RCEIITIGDGRAGDPFYHKLSELQGC	34.3	36.4	24.1	1.5
B6H β -3-B10-IR	CSVMTESKNDPDPFYHKLSELQGC	38.1	30.9	18.4	1.7
B6H β -3-B9-IR	GGEARRQOVGTANDPFYHKLSELAFGR	32.3	36.5	22.8	1.6
B6H β -3-D6-IR	GCAVTAITINGSDPFYHKLSELQGS	38.6	38.5	20.8	1.9
B6H β -3-C7-IR	GSKVKAMAVGTSDPFYHKLSELVQGR	35.9	36.0	15.6	2.3
	RCKGIKMSDDNDPFYHKLSELQCGG	38.3	38.0	6.6	5.8

FIGURE 2H (Con't)

Clone	Sequence	Ratios over Background		Comparisons	
Design		E-Tag	IGFsR	IGFR/IR	IR/IGFR
B6H-3-F1-IGFR	OOUUUUUUJJJJDPFYHKLSELXOO	--	---	--	--
B6H-3-F1-IGFR	RRVAAVA?KATGDGDPFYHKLSELRLSG	20.0	30.8	--	--
B6H-3-F1-IGFR	RSTMKEKTEGNDGDPFYHKLSELRLSG	19.0	27.6	--	--
B6H-3-F1-IGFR	GGAVIVTAARRGSDPFYHKLSELVGRG	14.2	25.2	--	--
B6H-3-F2-IGFR	SREAVEVTNARGSDPFYHKLSELVWGS	12.5	24.8	--	--
B6H-3-F2-IGFR	RSTTMKAVPPRPDPFYHKLSELL*GG	20.0	24.2	--	--
B6H-3-F3-IGFR	GRTEVVVGVTRRPDPFYHKLSELL*GG	14.2	22.8	--	--
B6H-3-F3-IGFR	RRMAGWQ*TSDDPFYHKLSELVSGS	13.0	22.8	--	--
B6H-3-F4-IGFR	SRKEVTEWVGSDPFYHKLSELMGSG	10.2	22.8	--	--
B6H-3-F4-IGFR	RGTAQRKSDP*DPFYHKLSELL*GG	14.0	22.5	--	--
B6H-3-F4-IGFR	GGVVAAGRRDPDPFYHKLSELVSGR	15.2	22.5	--	--
B6H-3-F4-IGFR	SR.MAMVEVGNPDGDPFYHKLSELLGS	14.5	21.9	--	--
B6H-3-F4-IGFR	RRVTAVIEVDGADDPFYHKLSELL*GG	11.6	21.8	--	--
B6H-3-F4-IGFR	RSVIAN??G?NADDPFYHKLSELL*GG	15.9	21.7	--	--
B6H-3-F4-IGFR	RGVVIETTKDGDGDPFYHKLSELL*GG	19.1	21.4	--	--
B6H-3-F4-IGFR	RRVTVMETVGRDPPFYHKLSELL*GG	11.3	20.9	--	--
B6H-3-F4-IGFR	GRVVAAAVRDPDPFYHKLSELVAGR	14.2	20.8	--	--
B6H-3-F4-IGFR	RGVATVVANHHSDPFYHKLSELVLRG	20.0	20.6	--	--
B6H-3-F4-IGFR	RRKMATIEMRSDADPFYHKLSELL*GG	12.5	20.3	--	--
B6H-3-F4-IGFR	GGKTAVEVTSPPDPFYHKLSELL*GG	12.1	19.3	--	--
B6H-3-F4-IGFR	RRKKVKVTTTNDGDPFYHKLSELVFGG	14.1	19.2	--	--
B6H-3-F4-IGFR	SSAIIMVADRADDPFYHKLSELL*GG	12.5	19.2	--	--
B6H-3-F4-IGFR	RRVAVIAAGAGDPPFYHKLSELL*GG	23.6	18.9	--	--
B6H-3-F4-IGFR	RRVMEAEENHADDPFYHKLSELL*GG	16.2	18.5	--	--
B6H-3-F4-IGFR	GRKMEIVAIRGADDPFYHKLSELL*GG	16.8	17.2	--	--
B6H-3-F4-IGFR	CCIAMVMAAGGDPFYHKLSELL*GG	14.6	17.1	--	--
B6H-3-F4-IGFR	RGAGSDPFYHKLSELL*GG	9.0	16.8	--	--
B6H-3-F4-IGFR	RKTAMVVIIGDASDPFYHKLSELL*GG	10.1	16.6	--	--
B6H-3-F4-IGFR	GSVITKAMKADGDPFYHKLSELL*GG	14.2	16.4	--	--

FIGURE 21

Clone	Design	Sequence	Ratios over Background		Comparisons	
			E-Tag	IR	IGFR/IR	IR/IGFR
B6H-4-D8-IGFR		OOUUUUUUUUJJJDDPFYHKLSLXXOO	--	--	--	--
B6H-4-D6-IGFR		GGVKAARERDDSPFYHKLSLFGS	15.1	16.4	--	--
B6H-4-E10-IGFR		CCEMVKTIHGGNDPFYHKLSLFGS	12.6	15.6	--	--
B6H-4-E10-IGFR		GGAKVAVVVDHDDPFYHKLSLFGS	10.2	15.1	--	--
B6H-4-E5-IGFR		RGKTMAAAGNRDPFYHKLSLFGN	12.3	14.8	--	--
B6H-4-B2-IGFR		GMATKIVTAPGHPFYHKLSLFGG	6.6	11.8	--	--
B6H-4-B2-IGFR		SGEGEMAMPDDPFYHKLSLFGSRA	8.2	11.6	--	--
B6H-3-F3-IGFR		GGMAEUVVVVGPDRDPFYHKLSLGGG	10.9	9.9	--	--
B6H-3-A2-IGFR		GGEVKVVADGTDPFYHKLSLGGT	5.9	9.6	--	--
B6H-3-H2-IGFR		SCVMVETVAGNRDPFYHKLSLVGGC	4.4	9.5	--	--
B6H-4-A1-IGFR		RRW*KVPOAADPFYHKLSLGRSA	7.2	8.7	--	--
B6H-4-C2-IGFR		GGVEATEVEHADGPFYHKLSLVGRS	6.7	8.6	--	--
B6H-4-H9-IGFR		RGVEVAVITHGPPDPFYHKLSLGRS	12.3	8.4	--	--
B6H-4-B7-IGFR		SGTVTVIAMSSTDPFYHKLSLGRS	6.4	8.2	--	--
B6H-4-A7-IGFR		GRTAVVKEASPAHDPFYHKLSLGRG	9.7	8.1	--	--
B6H-4-B3-IGFR		RGAI GNAAVGNRSDPFYHKLSLGRG	4.4	7.8	--	--
B6H-4-B4-IGFR		GMN1KTAMEHTRDPPFYHKLSLGRG	5.2	7.4	--	--
B6H-4-E1-IGFR		GCAEEVEVAGADHPFYHKLSLCAAG	3.6	7.1	--	--
B6H-3-C1-IGFR		SSVVVEVVADARRDPFYHKLSLV7SG	5.7	4.6	--	--
B6H-4-A3-IGFR		GRKAVATMTDGGDPFYHKLSLGRS	4.4	4.2	--	--
B6H-4-H10-IGFR		RGETENAVAUTDDDPFYHKLSLGRG	4.4	3.2	--	--
B6H-3-G1-IGFR		QQRDPFYHKLSLGRGA	2.4	2.9	--	--

FIGURE 21 (Cont'd)

Clone Design	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGFsR	IR	IGF/IR	IR/IGF	IR
B6C-3-C11-IR	EHNTVDPFYHKLSELLRESG	--	--	--	--	--	--
B6C-3-C4-IR	EHNTVDPFYD*ISELLRESG	33.4	35.0	36.7	1.0	1.0	1.0
B6C-3-B6-IR	EHNTVDPFYHLLQELLRESG	34.5	35.2	36.8	1.0	1.0	1.0
B6C-3-B6-IR	EHNTVDPFYHMLQELLRESG	34.2	36.1	36.9	1.0	1.0	1.0
B6C-4-E5-IR	EHNTVDPFYH*MSOLLRESG	35.5	35.8	36.9	1.0	1.0	1.0
B6C-4-H12-IR	EHNTVDPFYHLLQELLRESG	37.0	36.2	37.1	1.0	1.0	1.0
B6C-4-F9-IR	EHNTVDSFYHLLQELLRESG	36.1	36.2	37.3	1.0	1.0	1.0
B6C-3-A6-IR	EHNTVDPFYQGSSELLRESG	35.8	38.0	37.4	1.0	1.0	1.0
B6C-3-D1-IR	EHNTVDPFYQALQELLRESG	36.2	37.8	37.5	1.0	1.0	1.0
B6C-3-D4-IR	EHNTVDPFYMLQELLRESG	35.9	36.4	37.5	1.0	1.0	1.0
B6C-3-C1-IR	EHNTVDPFYHLLQELLRESG	36.8	36.6	37.9	1.0	1.0	1.0
B6C-4-G5-IR	EHNTVDPFYHLLQELLRESG	36.5	37.9	38.0	1.0	1.0	1.0
B6C-3-A8-IR	EHNTVDPFYHLLQELLRESG	34.4	37.0	38.1	1.0	1.0	1.0
B6C-4-H1-IR	EHNTVDPFYH*MSOLLRESG	36.8	36.5	38.3	1.0	1.0	1.0
B6C-3-D10-IR	EHNTVDPFYHMSOLLRESG	37.0	37.0	38.5	1.0	1.0	1.0
B6C-3-D12-IR	EHNTVDPFYQGLFELLRESG	36.2	37.0	38.7	1.0	1.0	1.0
B6C-3-B9-IR	EHNTVDPFYHLLQELLRESG	36.3	37.3	38.8	1.0	1.0	1.0
B6C-4-H7-IR	EHNTVDPFYH*MSOLLRESG	37.5	38.0	39.1	1.0	1.0	1.0
B6C-3-D11-IR	EHNTVDPFYHGL*ELLRESG	36.1	37.5	39.2	1.0	1.0	1.0
B6C-4-F10-IR	EHNTVDPFYHLLQELLRESG	37.9	38.6	39.3	1.0	1.0	1.0
B6C-4-G8-IR	EHNTVDPFYD*ISDLLRESG	35.9	38.0	39.7	1.0	1.0	1.0
B6C-3-A9-IR	EH*NTVDPFYHGLYELLRESG	36.5	38.8	39.9	1.0	1.0	1.0
B6C-3-A7-IR	EHNTVDAFYHGLQELLRESG	38.1	39.4	40.2	1.0	1.0	1.0
B6C-4-F12-IR	EHNTVDPFYQGLIELLRESG	38.0	38.4	40.2	1.0	1.0	1.0
B6C-4-G9-IR	EHNTVDPFYH*MSOLLRESG	37.5	39.4	40.5	1.0	1.0	1.0
B6C-4-H8-IR	EHNTVDPFYHLLQELLRESG	38.5	40.0	40.8	1.0	1.0	1.0
B6C-3-B10-IR	EHNTVDPFYQGLDLLRESG	39.3	40.3	40.9	1.0	1.0	1.0
B6C-3-A10-IR	EHNTVDPFYHGLQELLRESG	38.4	40.9	41.6	1.0	1.0	1.0
B6C-3-A3-IR	EHNTVDPFYH*MSOLLRESG	39.2	40.0	41.7	1.0	1.0	1.0
B6C-3-A5-IR	EHNTVDPFYHGLQELLRESG	38.2	40.4	41.9	1.0	1.0	1.0
B6C-3-C3-IR	EHNTVDPFYHMLQKLLRESG	34.5	34.6	32.0	1.1	0.9	0.9

FIGURE 2J (Cont)

Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IR	IGFR/IR	IR/IGFR
20E2A-3-B3-IR	XXXXXXFYDAIDQLVXXXXX	--	--	--	--
20E2A-4-H10-IR	RSSQSFYDAIEQLVLGGTCG	22.4	18.6	15.9	1.2
20E2A-4-F8-IR	VSRKSFYDAIEQLVLGGTCG	24.8	24.5	21.2	1.2
20E2A-4-H5-IR	FGTWSFYDAINQLVMEGSGD	4.3	2.2	2.1	1.1
20E2A-4-G1-IR	RGSAIFYDAINQLVQDGGW	21.3	18.3	16.5	1.1
20E2A-4-F2-IR	AQPCVSFYDAIEQLVTRSCM	21.4	18.3	16.0	1.1
20E2A-4-H7-IR	GGDGPFWIEQLVVRAGSEA	20.1	20.5	18.2	1.1
20E2A-3-D5-IR	LDLCASFYDAIEQLVGVKFCG	22.6	21.2	18.6	1.1
20E2A-3-A1-IR	WLACQSFYDAIDQLVGGECN	22.7	21.3	18.9	1.1
20E2A-4-H9-IR	EVNALSFYDAIDQLVGGELG	23.8	21.7	19.9	1.1
20E2A-4-B5-IR	RLQPRTFYDAIDQLVGVLEG	24.0	22.5	20.8	1.1
20E2A-4-E11-IR	SGAHRIFYDAIEQLVGMGSK	24.1	23.5	21.0	1.1
20E2A-4-B4-IR	NNQSLTFYDAIEQLVGMGSG	24.1	23.5	20.9	1.1
20E2A-4-G8-IR	RAVGATFYDAINQLVKDDGY	22.5	14.6	11.7	1.3
20E2A-4-G11-IR	SQCRGGFYDAIEQLVGVNCCI	20.2	17.5	13.4	1.3
20E2A-4-G6-IR	DLAFSFYDAIDQLVHCCHG	21.7	18.0	13.8	1.3
20E2A-3-B7-IR	GNRQGFYDAIDQLVGGWNR	21.1	21.4	16.9	1.3
20E2A-4-G11-IR	GGVLSFYDAIEQLVGGQSI	22.9	23.1	17.7	1.3
20E2A-4-B6-IR	RSGPMSFYDAIEQLVGLRLHP	24.2	24.3	19.0	1.3
20E2A-4-H2-IR	VSGCRIFYDAIEQLVSGQCG	17.1	11.5	9.4	1.2
20E2A-4-H4-IR	AQFPRIFYDAIEQLVHGKMD	21.6	13.7	11.6	1.2
20E2A-4-F5-IR	CAQPSFYDAIDRLVTRCLV	21.3	19.6	16.3	1.2
20E2A-3-B10-IR	PDEQSFYCAIDRLVTKGGR	23.2	22.2	18.0	1.2
20E2A-3-B9-IR	PLVRCTFYDAIEQLVGMGSSD	12.2	5.7	3.8	1.5
20E2A-3-D10-IR	PLVRCTFYDAIEQLVGMGSSD	14.9	5.9	3.9	1.5
20E2A-3-D6-IR	PRQASFYDAIEQLVGSADWN	15.5	11.0	7.2	1.5
20E2A-4-G9-IR	DGRVMSFYDAIEQLVQGFEP	22.2	19.1	12.8	1.5
20E2A-4-E1-IR	RFVRSFYDAIEQLVLPNLG	21.8	19.3	13.0	1.5
20E2A-4-F12-IR	KVGRSFSYDAIEQLVGGGHV	21.3	19.9	13.3	1.5
20E2A-4-G3-IR	PAIGFTFYDAIEQLVWFQGD	23.1	20.7	13.6	1.5
	ALPGRSFYDAIEQLVGFDMGA	17.5	17.1	12.1	1.4
		21.6	19.4	14.1	0.7

FIGURE 21 (Con't)

Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR IR/IGFR
20E2A-3-C2-IR	XXXXXXFYDAIDQLVXXXXX	23.4	20.9	15.4	1.4 0.7
20E2A-3-B1-IR	RPQGGTFYDMIKQLVGGWG	22.1	21.6	15.6	1.4 0.7
20E2A-3-A8-IR	WSAFADFYDAIQHLVAGEVA	12.3	4.8	2.7	1.8 0.6
20E2A-3-A8-IR	SDGRDGFYDAIQLVRSFGD	18.9	13.8	7.9	1.8 0.6
20E2A-4-G2-IR	IRSVFSFYDAIDQLVKGWGS	23.3	20.3	11.3	1.8 0.6
20E2A-3-A9-IR	GVSLTFYEALQVRRGGFDA	24.4	24.5	13.5	1.8 0.6
20E2A-3-D3-IR	AAQAFSYDLINQVASKPSE	13.5	4.6	2.7	1.7 0.6
20E2A-3-A11-IR	QSGACGFYDAINQLVWSIC	21.4	15.3	8.9	1.7 0.6
20E2A-3-B4-IR	GGTVFSFYDAIDQLVRNGAG	22.3	19.0	11.3	1.7 0.6
20E2A-4-E10-IR	IYTGQGFYDAIEQLVRGGSTP	22.5	19.0	11.2	1.7 0.6
20E2A-3-D2-IR	KSPALSFYDAIEQLVGSQVR	14.5	6.2	3.9	1.6 0.6
20E2A-4-F1-IR	ISPPWTFYDAIDQLVGGSDGR	16.5	6.6	4.0	1.6 0.6
20E2A-3-D1-IR	GSRRGFYDAIDQLVRQGGL	20.2	14.4	8.9	1.6 0.6
20E2A-3-D11-IR	GVAGGTFYDAIEQLVRQFGGS	23.9	22.5	14.2	1.6 0.6
20E2A-3-C3-IR	RPLRWSFYDALDQLVGSALGG	21.3	23.0	14.4	1.6 0.6
20E2A-3-C12-IR	MQCRGGFYDAIALVGGHVRG	18.9	11.6	7.5	1.5 0.6
20E2A-3-A2-IR	TSQGLSFYDAINQLVAGGWWG	21.6	15.1	6.9	2.2 0.5
20E2A-3-C7-IR	SGGTTFYDAINQLVQGRYNG	18.1	18.0	9.1	2.0 0.5
20E2A-3-C10-IR	GGALDPFYDAINQLVIRGSSG	21.8	21.6	8.4	2.6 0.4
20E2A-3-D9-IR	KQRGVTFYDLINQLVGSARG	24.3	18.1	7.4	2.5 0.4
20E2A-3-B5-IR	PRAPRSFYDAIHQLVRRQPGC	17.8	19.1	7.6	2.5 0.4
20E2A-3-A6-IR	PCSDQGFYDALSLQVIRVCP	9.0	9.3	2.6	3.6 0.3
20E2A-4-G12-IR	SYGYQSFYDAIEELVRGPPAR				

FIGURE 2L (Cont.)

Clone Design	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGF5R	IR	IGF5R/IR	IR/IGF5R	
Parental	XXXXXXFYDAIDQLVXXXXX	--	--	--	--	--	--
20E2A-4-F11-IGFR	FYDAIDQLVRSARAGTRD	30.6	15.1	4.2	3.6	0.3	
20E2A-4-F12-IGFR	QGSASFYDAIDRLRMRIIG	21.3	18.8	1.3	14.6	0.1	
20E2A-3-B4-IGFR	AQSSGFYDALAQVLVSG	23.3	23.9	3.1	7.8	0.1	
20E2A-4-F4-IGFR	GHPAVSFYDAIDQLRRGGG	21.8	16.6	2.4	6.9	0.1	
20E2A-3-C7-IGFR	YSDTSFYDAIQVLRGASA	20.7	20.0	3.6	5.5	0.2	
20E2A-3-C10-IGFR	VGTVAGFYDAIQVLRASRV	17.6	5.4	1.1	5.1	0.2	
20E2A-3-D6-IGFR	RFVWSFYDAIDQLVQWRHG	23.3	21.0	4.2	5.0	0.2	
20E2A-4-F6-IGFR	RAVGSFYDAIQVLRGGHV	15.1	11.8	2.4	5.0	0.2	
20E2A-3-A8-IGFR	LRQLSFYDAIDQLVQWKGGA	21.5	19.9	4.3	4.6	0.2	
20E2A-4-F9-IGFR	DKFTSFYDAIDQLVQVRGV	22.2	13.3	2.9	4.6	0.2	
20E2A-4-F3-IGFR	MQSGSFYDAIDRLVRLGR	21.2	19.0	4.4	4.4	0.2	
20E2A-3-B2-IGFR	VGSSSFYDAIERLVQCLGRH	20.6	19.3	4.6	4.2	0.2	
20E2A-4-G8-IGFR	LSWAAGFYDAIDQLVRSRGR	18.7	14.7	3.8	3.9	0.3	
20E2A-3-D10-IGFR	QVHAGFYDAIEELVGFGLG	20.9	10.8	2.7	3.9	0.3	
20E2A-3-A12-IGFR	MMVVDGFYDALHQLVVAQSLG	20.6	6.9	1.8	3.9	0.3	
20E2A-3-A11-IGFR	LSVALSFYDALGOLVAGEGRW	16.1	4.3	1.1	3.9	0.3	
20E2A-4-H1-IGFR	SGSNLGFYDALGOLVATDGS	17.8	9.7	2.6	3.7	0.3	
20E2A-4-F7-IGFR	PSGFLSFYDAIDQLVHVRWF	20.8	14.5	4.1	3.5	0.3	
20E2A-3-D7-IGFR	AFTPTSFYDAIEQLVQLSR	19.5	17.9	5.3	3.4	0.3	
20E2A-3-A9-IGFR	VSSLASFYDALDELVRPFQO	22.0	18.3	5.6	3.3	0.3	
20E2A-3-A10-IGFR	VSMQSFYDALKOLVRISGG	24.7	10.5	3.2	3.3	0.3	
20E2A-3-B11-IGFR	IGVSRGFYDAIDKLVRDRGSP	26.3	15.4	4.8	3.2	0.3	
20E2A-3-D12-IGFR	GRSLLSFYDALDQLVQAGNGG	15.8	10.7	3.4	3.2	0.3	
20E2A-4-H11-IGFR	QRQAQSFYDALRLVCEGRCT	13.9	9.0	2.8	3.2	0.3	
20E2A-4-H5-IGFR	CRFQGSFYDAIDLLVLRVTC	22.8	17.5	5.7	3.1	0.3	
20E2A-4-E11-IGFR	RMAQSFYDAIDHLVNNRHHG	20.1	16.6	5.5	3.0	0.3	
	LPSSGCFYDAIQVLCGRGC	21.0	12.6	4.2	3.0	0.3	

FIGURE 2M

Clone Design	Sequence	Ratios over Background			Comparisons		
		E-Tgr	IR	IGFR	IGFR/IR	IR/IGFR	
20E2A-4-F2-IGFR	XXXXXXFYDAIQDLVXXXXXX	--	--	--	--	--	--
20E2A-3-D4-IGFR	TGVNFDFYDAIQDLVGRVSD	19.4	19.0	6.8	2.8	2.8	0.4
20E2A-3-D4-IGFR	YGSFETFYDAIQDLVRRGSQF	16.1	11.8	4.2	2.8	2.8	0.4
20E2A-3-B10-IGFR	ROLLDSFYDAIQDLVRSERF	24.0	14.3	5.3	2.7	2.7	0.4
20E2A-4-E5-IGFR	WPGDPFYDAIMEKLLSQGGR	18.1	20.6	7.9	2.6	2.6	0.4
20E2A-3-D5-IGFR	PGLQSFYDAIQDLVQRGN	15.1	9.3	3.6	2.6	2.6	0.4
20E2A-4-G11-IGFR	MMVFSFYDAIQDLVQCRIGC	20.7	3.3	1.3	2.6	2.6	0.4
20E2A-3-C12-IGFR	LMVGGFYDAIQDLVSGSLAP	25.9	17.4	7.2	2.4	2.4	0.4
20E2A-4-G5-IGFR	RRPNSFYDAIQDLVGGPCG	23.6	14.6	6.0	2.4	2.4	0.4
20E2A-3-D9-IGFR	FGRRSTFYDAIQDLVQGRGT	19.8	12.3	5.1	2.4	2.4	0.4
20E2A-4-F10-IGFR	LRAPRSFYDAIQDLVQRPSP	21.4	21.6	9.3	2.3	2.3	0.4
20E2A-4-E2-IGFR	VQRFSSFYDAIQDLVGHVWK	22.6	21.3	9.1	2.3	2.3	0.4
20E2A-4-E3-IGFR	PGARMFYDAIQDLVGLVPGS	21.0	21.8	10.1	2.2	2.2	0.5
20E2A-3-C4-IGFR	SLQPHDFYDAIHLVFGHGRF	23.5	17.4	7.8	2.2	2.2	0.4
20E2A-3-C5-IGFR	ERHGGSFYDAIQDLVQSDRSR	22.2	17.1	7.7	2.2	2.2	0.4
20E2A-4-G12-IGFR	YQPGSFYDAIHLVAGPRRE	24.3	16.3	7.4	2.2	2.2	0.5
20E2A-3-C3-IGFR	FAHASSFYDAIQDLVAKQSP	11.3	2.7	1.2	2.2	2.2	0.5
20E2A-3-B7-IGFR	AQSSGSFYDAIQDLVWGRGPG	22.3	22.6	10.8	2.1	2.1	0.5
20E2A-4-E10-IGFR	TTSGGSFYDAIQDLVWGDWNR	22.6	19.9	9.4	2.1	2.1	0.5
20E2A-4-E6-IGFR	ARGTAGFYDAIHLVQGDHGG	23.0	16.6	7.9	2.1	2.1	0.5
20E2A-3-D3-IGFR	PRHAINFYDAIQDLVFGPRQ	20.5	15.7	7.6	2.1	2.1	0.5
20E2A-4-G4-IGFR	QSAHWSFYDAIHLVNMDDTP	22.6	14.5	7.0	2.1	2.1	0.5
20E2A-4-F1-IGFR	VGVWSSFYDAIQDLVWDRGNS	19.6	19.9	9.8	2.0	2.0	0.5
20E2A-4-H7-IGFR	DTLIASFYDAIQDLVRLGRNQ	23.0	17.1	8.7	2.0	2.0	0.5
20E2A-3-B9-IGFR	FQGTQGFYDAIHLVRRGERP	26.4	22.1	11.5	1.9	1.9	0.5
20E2A-4-E9-IGFR	WADGWSFYDAIQDLVQRGGV	25.3	20.7	11.1	1.9	1.9	0.5
20E2A-3-B1-IGFR	EQLSGCFYDAIQDLVHGGLG	23.1	17.9	9.5	1.9	1.9	0.5
20E2A-4-G1-IGFR	CQGRCSFYDAIQDLVHLLPGA	22.6	17.7	9.3	1.9	1.9	0.5
20E2A-4-E7-IGFR	MMRVDFYDAIHLVNEGGQAT	17.2	8.6	4.6	1.9	1.9	0.5
20E2A-3-D11-IGFR	RQATSFYDAIQDLVGGSGGV	16.1	6.1	3.2	1.9	1.9	0.5
20E2A-3-A3-IGFR	GHYGSFYDAIQDLVACMLPG	5.2	3.0	1.5	1.9	1.9	0.5
20E2A-4-G7-IGFR	PEGVQGFYDALAHVGGSLFG	24.4	21.1	11.5	1.8	1.8	0.5

FIGURE 2M (Con't)

Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tgr	IGFSR	IGFSR/IR	IR/IGFSR
20E2A-4-G2-IGFR	XXXXXXFYDAIQDLVXXXXXX	--	--	--	--
20E2A-4-G3-IGFR	IGVLGSFYDAIQDLVRQGNR	22.3	17.5	9.9	1.8
20E2A-4-G3-IGFR	RDVADGYAAIQDLVRQFGL	21.2	12.3	6.9	1.8
20E2A-3-B5-IGFR	VRQAKSFYDAIQDLVRGALRG	24.0	22.7	13.3	1.7
20E2A-4-H4-IGFR	QVFRGSFYDAIQDLVRGGBA	22.2	20.6	12.0	1.7
20E2A-4-F8-IGFR	GVAAFSFYDAIQDLVRSFHS	17.3	17.9	10.7	1.7
20E2A-3-A5-IGFR	PSFVMSFYDAIQDLVRSQGR	23.8	23.7	15.0	1.6
20E2A-4-H12-IGFR	PVSATSFYDAIQDLVRSQGR	25.1	23.5	14.2	1.6
20E2A-3-B12-IGFR	VMRDRFYDAIQDLVGGRIQV	27.6	21.9	13.6	1.6
20E2A-3-B8-IGFR	TYVNSFYDAIQDLVGGADV	21.5	19.0	12.2	1.6
20E2A-3-C8-IGFR	LSNMITYDAIQDLVGHVQSL	23.2	17.7	11.4	1.6
20E2A-4-H10-IGFR	ASSRLSFYDAIQDLVWSPQ	25.3	23.8	16.2	1.5
20E2A-3-C9-IGFR	WDLVDSFYDAIQDLVQVRPG	25.4	21.8	14.6	1.5
20E2A-4-H2-IGFR	FAFGSFYDAIQDLVQGRPS	21.8	20.1	13.0	1.5
20E2A-3-B6-IGFR	EDQPNFSFYDAIQDLVGRISP	20.3	18.1	11.8	1.5
20E2A-4-G9-IGFR	SVGPRSFYDAIQDLVGGAWG	26.0	16.1	10.8	1.5
20E2A-4-H6-IGFR	KFRVYTFYDAIQDLVWQGR	21.9	19.6	13.9	1.4
20E2A-4-H9-IGFR	GRGWSFYDAIQDLVRLGET	24.9	16.8	11.8	1.4
20E2A-4-G10-IGFR	FTSPHTFYDAIQDLVGGQDP	25.3	16.5	12.1	1.4
20E2A-3-A4-IGFR	AGSVTSFYDAIQDLVATGCTSA	16.8	2.5	1.8	0.7
20E2A-3-A7-IGFR	PRESFSFYDAIQDLVGRVRS	26.0	24.9	19.3	1.3
20E2A-4-E12-IGFR	LGKADGFYDAIQDLVGDWGG	23.3	23.1	17.8	1.3
20E2A-3-D1-IGFR	RSGTWTFYDAIQDLVQSGSR	24.0	22.4	17.6	1.3
20E2A-3-C6-IGFR	PVVLF SFYDAIQDLVRKGLGP	23.7	21.7	17.2	1.3
20E2A-3-D2-IGFR	GRAGTFYDAIQDLVGGEGALG	21.4	15.1	11.4	1.3
20E2A-4-E8-IGFR	AGPMSFYDAIQDLVHCCGPF	18.4	13.6	10.4	1.3
20E2A-4-G6-IGFR	HGEKLSFYDAIQDLVGFDTGH	24.7	21.9	17.7	1.2
20E2A-4-F5-IGFR	GYPVDFYDAIQDLVGTGMPG	21.7	17.2	18.2	1.2
20E2A-3-C2-IGFR	FGFSFSFYDAIQDLVARGSD	22.5	19.6	15.8	1.2
20E2A-4-H8-IGFR	VGLVRGFYDAIQDLVGDTHQ	24.4	18.5	15.1	1.2
20E2A-3-A5-IGFR	TPGFSFYDAIQDLVQDVLSDS	22.7	15.6	12.6	1.2
20E2A-3-C11-IGFR	TNAALTFYDAIQDLVWRGQRD	25.8	24.3	21.2	1.1

FIGURE 2M (Cont.)

Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGfR	IGfR/IR	IR/IGfR
20E2A-3-C1-IGFR	XXXXXXXXFYDAIDQLVXXXXXX	--	--	--	--
20E2A-3-B3-IGFR	GSPLSFYDAIDQLVRAFPVG	23.4	22.4	20.5	1.1
20E2A-3-D8-IGFR	AGQLGGFYIAICQLVGEYCT	21.0	17.0	14.8	1.1
	SAGPLSFYDAIAQLVGPANRL	22.0	19.7	19.6	1.0

FIGURE 2M (Con't)

Clone Design	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGF5R	IR	IGFR/IR	IR/IGFR	
Parental	XXXXXXXXXXXXXXXXXXXX	--	--	--	--	--	--
20E2Ba-3-B3-IR	FYDAIDQLVRSRAGGTRD	30.6	15.1	4.2	3.6	0.3	
20E2Ba-4-F12-IR	AGVNAGFYRYFSTLLDWDQ	33.5	1.2	23.5	0.1	20.0	
20E2Ba-3-B8-IR	SVKEVQFYRYFDLLQSEESG	35.5	5.9	27.8	0.2	4.7	
20E2Ba-3-D2-IR	IEVTQFYDYFQQLRLYGND	39.3	18.2	36.5	0.5	2.0	
20E2Ba-3-A5-IR	VQCRADFYSPFACLVGRFCGR	42.6	19.7	26.7	0.7	1.4	
20E2Ba-3-A3-IR	RNYPIGFYFPHFELVISGGG	36.9	22.7	24.5	0.9	1.1	
20E2Ba-4-E9-IR	DLGNSFYVGLRLVLQDAVG	39.9	33.5	35.5	0.9	1.1	
20E2Ba-4-G8-IR	CKQDPDFYMGIKCLISGGGV	32.8	29.6	28.6	1.0	1.0	
20E2Ba-4-F9-IR	ACEGSGFYCLQSLMSVESGN	37.5	30.5	30.9	1.0	1.0	
20E2Ba-4-E7-IR	AVHEDGFYDMLKLLSBDGS	35.6	32.5	31.1	1.0	1.0	
20E2Ba-3-D5-IR	LAHNEFYRYFEQLVFGDTG	36.0	31.6	31.2	1.0	1.0	
20E2Ba-4-F7-IR	ATCASSFYAQLNLLSDFVM	39.5	33.1	31.8	1.0	1.0	
20E2Ba-3-B12-IR	VQACQNFYDCLNTLLLLDLOG	36.6	32.9	32.5	1.0	1.0	
20E2Ba-3-A11-IR	IRGADQFYQFRELLESVGE	37.0	33.4	33.5	1.0	1.0	
20E2Ba-3-B7-IR	RAGSRGFYEFENLLRVGAGS	36.9	34.9	34.2	1.0	1.0	
20E2Ba-3-B5-IR	AQCRADFYACIELLIAPGSMR	40.4	37.1	36.3	1.0	1.0	
20E2Ba-4-G1-IR	PGGGGFGYQLQLRLILGADGG	41.6	36.4	34.5	1.1	1.0	
20E2Ba-4-G11-IR	QKSEAFYDMLADLLQGETSG	38.5	28.9	26.5	1.1	0.9	
20E2Ba-4-E10-IR	WGLRDDFYRGIRCLVQWSEGC	33.2	30.1	27.8	1.1	0.9	
20E2Ba-4-F11-IR	DSTVCGFYCRLAQLVAREGSP	35.4	30.5	28.0	1.1	0.9	
20E2Ba-4-H11-IR	QHSCTFYDCIRVMDGQQLG	32.5	29.5	28.0	1.1	0.9	
20E2Ba-4-H3-IR	WGNVDFYIMIRQLCGVCGS	34.8	32.0	28.7	1.1	0.9	
20E2Ba-4-H7-IR	QTVHRDFYAALQDLLINDLGF	38.7	34.9	30.5	1.1	0.9	
	SSCCDFYSCMIQLVTTGGGD	35.3	32.5	30.5	1.1	0.9	

FIGURE 2N

Clone Design	Sequence	Ratios over Background				Comparisons			
		E-Tag	IGF5R	IR	IR	IGF5R	IR	IGF5R	IR/IGF5R
20E2Ba-3-B6-IR	XXXXXXFYxxxhxxhxxxxxx	--	--	--	--	--	--	--	--
20E2Ba-3-D1-IR	SGPMVGYRGLFSLSPEDLQ	39.7	34.9	31.5	31.5	1.1	1.1	1.1	0.9
20E2Ba-3-A9-IR	LAEPDSFYNNIAQLLEEGPAG	41.6	35.1	31.7	31.7	1.1	1.1	1.1	0.9
20E2Ba-3-A9-IR	FSGCDNFYSCIQSLWLPQGV	37.3	35.1	32.4	32.4	1.1	1.1	1.1	0.9
20E2Ba-3-C4-IR	QVFCDFYHCITLWVGQTP	39.6	36.3	33.4	33.4	1.1	1.1	1.1	0.9
20E2Ba-4-F3-IR	RGDNQFYHGLWALLGSGLE	37.5	36.6	33.6	33.6	1.1	1.1	1.1	0.9
20E2Ba-4-F4-IR	VSRGGFYDAIRDLI GPRDQG	37.2	36.9	33.7	33.7	1.1	1.1	1.1	0.9
20E2Ba-3-D4-IR	PVLLDDFYVALCQLMWGQDGF	42.1	38.0	34.5	34.5	1.1	1.1	1.1	0.9
20E2Ba-4-E4-IR	PDIADPFYAFQGLRADTPI	40.6	38.4	35.5	35.5	1.1	1.1	1.1	0.9
20E2Ba-4-G10-IR	VAQCTDFYACIRSLVRSGSPG	32.9	31.3	27.1	27.1	1.2	1.2	1.2	0.9
20E2Ba-3-D11-IR	CSQLVSYFLGMDCLLGGGTQ	34.0	32.5	27.9	27.9	1.2	1.2	1.2	0.9
20E2Ba-3-C8-IR	PLACADFYQLSDLI RGGPAW	39.2	33.0	28.2	28.2	1.2	1.2	1.2	0.9
20E2Ba-4-F2-IR	VVICTFYDCIYQLVGSHEEM	38.7	37.6	32.3	32.3	1.2	1.2	1.2	0.9
20E2Ba-4-H12-IR	CVDRRTFYBGLQCLVGATGD	32.3	30.4	25.8	25.8	1.2	1.2	1.2	0.8
20E2Ba-4-E1-IR	VNLRDPFYOWIEALMDSAGGE	39.2	40.2	32.3	32.3	1.2	1.2	1.2	0.8
20E2Ba-4-H8-IR	LTSTSFYDALFCLAGLQICG	37.6	34.8	27.0	27.0	1.3	1.3	1.3	0.8
20E2Ba-3-B4-IR	DFDSPPFYGLRFLLESFPP	39.9	34.9	25.9	25.9	1.3	1.3	1.3	0.7
20E2Ba-4-E2-IR	HEAGWTFYDAIQCLVGMGCSK	38.8	36.3	23.5	23.5	1.5	1.5	1.5	0.6
20E2Ba-4-H1-IR	CQWRSPFYHANSCLLGDDPD	40.8	33.6	20.2	20.2	1.7	1.7	1.7	0.6
20E2Ba-3-A10-IR	MVDRDPFYQLRDLI GRQSKG	32.8	32.6	18.5	18.5	1.8	1.8	1.8	0.6
20E2Ba-3-D3-IR	LGRRCGFYRGLQDLIGTQWPR	41.9	29.5	5.6	5.6	5.3	5.3	5.3	0.2

FIGURE 2N (Cont'd)

Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IR	IGFR/IR	IR/IGFR
20E2B-1-A6-IGFR	XXXXXXFYXXhXhXhXXXXXX	--	--	--	--
20E2B-3-C6-IGFR	GVAMSFYDALNVSLVGLPSG	18.6	18.1	1.1	16.8
20E2B-4-H3-IGFR	VEGGLFYDLQLLARRQNG	17.9	16.8	1.1	14.8
20E2B-3-C2-IGFR	KLHNLFFYGLQLRVGNGLG	11.2	14.8	1.1	13.9
20E2B-3-E3-IGFR	GNQDMFYGLSLLVGRDMHV	13.1	8.9	0.6	13.8
20E2B-4-H12-IGFR	PDHKGFFAQLIRQLQLLS	22.4	16.3	1.3	13.1
20E2B-3-D2-IGFR	YSCDGFYSLLSDLLGGQPRC	6.5	9.7	0.8	12.8
20E2B-3-D8-IGFR	IQELTFYDLHLVRSELGS	20.7	12.4	1.1	11.7
20E2B-3-E8-IGFR	GTETDFYRALERLVRCGLG	20.4	17.7	1.6	11.3
20E2B-4-F8-IGFR	LRIANLFYQRLMDLAFGGG	15.7	16.7	1.5	11.1
20E2B-1-A11-IGFR	PVGQGFYGLSRLVLRGCG	12.3	7.3	0.8	9.7
20E2B-3-D4-IGFR	RFSTDGFYQLLALVGGPGV	15.0	9.5	1.0	9.7
20E2B-2-B11-IGFR	NSRDGFFYGLQLERLLGFVVG	8.1	7.9	0.8	9.6
20E2B-3-C8-IGFR	VTFPVNFYRALERLVRCRLG	13.9	10.6	1.1	9.4
20E2B-2-B2-IGFR	QAPDGFYSALMKLIGRGVVS	18.5	15.6	1.8	8.9
20E2B-4-F10-IGFR	PETDLGFYQALRCVVIQACD	11.7	4.9	0.6	8.1
20E2B-3-F9-IGFR	AQPCGFFYGLQLLVGRSVC	19.0	17.3	2.2	7.8
20E2B-3-D11-IGFR	QPDHSYFYSLLQELVGSERL	11.9	14.7	1.9	7.7
20E2B-3-C11-IGFR	LGVTDGFYALGYLHGVQGF	14.3	12.2	1.6	7.6
20E2B-2-B3-IGFR	CMWQDGFYALGLCLTAGBGR	15.3	15.4	2.1	7.5
20E2B-3-D12-IGFR	ICTGGFFYQVLCLLRTGSAR	9.1	5.3	0.7	7.4
20E2B-3-E12-IGFR	QGNVLDYFYMIGRLIAKQSD	10.3	6.2	0.9	7.3
20E2B-2-B8-IGFR	VATSGFFYGLSELLQGGNV	13.9	6.0	0.8	7.3
20E2B-4-G11-IGFR	INATGDFYLLSGLVMGRVGT	17.4	5.7	0.8	7.2
20E2B-3-D6-IGFR	RQSGFYMLMLSLVAVGARG	8.9	4.5	0.6	7.0
20E2B-2-B7-IGFR	DSVGNFYQLLESILVGGHGV	20.7	17.8	2.6	6.9
20E2B-3-C4-IGFR	LSSDGFYRALNLLIQSAGR	18.0	6.1	0.9	6.7
	ASSAGFFYELLQRLAGLGLGV	23.4	20.4	3.3	6.2

FIGURE 20

Clone Design	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGFR	IR	IGFR/IR	IR/IGFR	
20E2B-3-D3-IGFR	XXXXXXXXXXhXXXXXXXX	21.0	16.1	2.6	--	--	0.2
20E2B-4-H8-IGFR	CGSRDFYGGICLLGQGVV	7.2	5.3	0.9	5.9	5.9	0.2
20E2B-3-E9-IGFR	PAGCGFYCGLLGLHGDQP	14.7	16.2	2.8	5.8	5.8	0.2
20E2B-4-H9-IGFR	QAAQDFYQGLMLLHRDPTM	4.5	5.2	0.9	5.6	5.6	0.2
20E2B-1-A8-IGFR	RCQGTGYTCIQELIGFGDP	16.1	4.4	0.9	5.0	5.0	0.2
20E2B-4-H11-IGFR	TLRSPTFYDMLWLVTHGQG	10.7	11.0	2.3	4.8	4.8	0.2
20E2B-3-C9-IGFR	STHSRAFYDAIAGLVGSVLP	17.9	19.7	4.2	4.6	4.6	0.2
20E2B-3-E6-IGFR	RQGGGFYELLCGLVGEVCV	24.5	21.6	4.7	4.6	4.6	0.2
20E2B-3-E11-IGFR	RQASGFYRALHDLMLRTDY	16.5	7.7	1.9	4.1	4.1	0.2
20E2B-4-G8-IGFR	SRANLIFYMGLSGLLRNRL	11.1	14.9	3.7	4.1	4.1	0.2
20E2B-4-H10-IGFR	GRALDPFYDQLRLVARSGG	2.2	2.5	0.8	3.1	3.1	0.3
20E2B-3-E7-IGFR	EASCRFYCGMLIGGDDGR	14.4	8.8	3.0	3.0	3.0	0.3
20E2B-3-C12-IGFR	QNGCKDFYCLINDLIRYPPG	6.2	6.4	2.2	2.9	2.9	0.3
20E2B-4-G12-IGFR	QHSCTFYDCIRVMDGQILG	9.6	10.0	3.8	2.6	2.6	0.4
20E2B-4-G3-IGFR	LPSRRGFYDMIKALIGDRVQ	27.2	23.8	9.1	2.6	2.6	0.4
20E2B-3-E4-IGFR	COQKGFYAGLVCLLRERASQ	24.9	22.3	8.9	2.5	2.5	0.4
20E2B-3-E2-IGFR	GSQGSFYDNCMLLQDPTC	18.6	20.2	8.7	2.3	2.3	0.4
20E2B-2-B4-IGFR	VESDVSFYEGMLRVWVGQGG	5.8	2.3	1.0	2.2	2.2	0.5
20E2B-3-C5-IGFR	VRSGDLFYQMFELVAGHLE	6.3	2.0	0.9	2.2	2.2	0.5
	RMPSGSFYQGIYELVTRQGF						

FIGURE 20 (Cont'l)

Clone Design	Sequence	Ratios over Background				Comparisons			
		E-Tag	IGFR	IR	IGFR	IGFR/IR	IR/IGFR	--	--
NRpβ-4-G6-IR	XXXXXXFYRYFXLLXXXXX	10.1	1.9	20.1	0.1	10.6	0.1	10.6	
NRpβ-4-F3-IR	RMNFYGVFESLLTHFS	8.6	1.3	13.6	0.1	10.5	0.1	10.5	
NRpα-2-C1-IR	HYNAFYEFQVLLAETW	19.7	2.0	10.9	0.2	5.3	0.2	5.3	
NRpα-4-E1-IR	EGWDFYSFGLIASVT	11.5	6.5	21.2	0.3	3.2	0.3	3.2	
NRpα-3-H6-IR	LDRQFYRYFQDLLVGFM	19.1	2.1	6.0	0.3	2.9	0.3	2.9	
NRpβ-4-F7-IR	WGRSFYRYFETLLAQGI	0.7	0.9	2.3	0.4	2.7	0.4	2.7	
NRpα-2-D1-IR	RREGFYHYFQSLLDYEG	18.4	1.5	3.7	0.4	2.5	0.4	2.5	
NRpα-1-A1-IR	GGQGFYRYFIDMLVDI	15.2	1.3	3.1	0.4	2.4	0.4	2.4	
NRpα-2-C10-IR	PTGPFDRYFARLLVWRG	18.8	3.8	8.8	0.4	2.3	0.4	2.3	
NRpα-3-G1-IR	RGGAFFRYFEGLLSQHN	18.9	4.2	8.6	0.5	2.1	0.5	2.1	
NRpα-4-C3-IR	WRDPFYRYFQDLLEGER	17.9	12.9	25.7	0.5	2.0	0.5	2.0	
NRpα-4-D1-IR	WGGEFYRYFVQLLSEED	16.2	12.7	23.2	0.5	1.8	0.5	1.8	
NRpβ-4-F4-IR	GRESFYGYFLDLLOETV	19.5	16.0	25.6	0.6	1.6	0.6	1.6	
NRpα-1-B2-IR	GHAEFYGYFQGLLSYL	14.8	8.4	12.9	0.7	1.5	0.7	1.5	
NRpα-1-B4-IR	GGEAFYRYFTGLLSDGQ	19.1	6.3	9.2	0.7	1.5	0.7	1.5	
NRpα-4-D9-IR	DPGAFYRYFAQMLMTWN	7.6	16.9	25.7	0.7	1.5	0.7	1.5	
NRpβ-4-F2-IR	KHEQFYRYFRNLLGAMS	21.6	20.9	30.8	0.7	1.5	0.7	1.5	
NRpβ-4-H12-IR	RDGAFFRYFEDLLIAND	5.2	13.8	20.0	0.7	1.5	0.7	1.5	
NRpα-4-E7-IR	RGNRFYRYFEYLLLDYG	9.4	21.9	29.7	0.7	1.4	0.7	1.4	
NRpα-1-B5-IR	ELGDFYRYFQLLLAADMH	14.1	5.4	7.1	0.8	1.3	0.8	1.3	
NRpα-4-C4-IR	AGDAFYYSFVULLGEHL	17.6	17.6	22.3	0.8	1.3	0.8	1.3	
NRpα-4-C7-IR	IGVNFYRYFEKLLIDEF	4.5	11.2	14.9	0.8	1.3	0.8	1.3	
NRpα-4-D3-IR	TDSQFYYSFESILLETFG	16.4	13.5	17.9	0.8	1.3	0.8	1.3	

FIGURE 2P

Clone Design	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFR	IR	IGFR/IR	IR/IGFR
NRRE β -4-G10-IR	xxxxxxfyrfxxllxxxxxx	8.8	8.7	11.7	0.7	1.3
NRRE β -4-H2-IR	ssrfyfysfgllttal	4.9	19.9	25.3	0.8	1.3
NRRE β -4-C1-IR	tgrgfyrfeegllldm	15.5	18.0	21.1	0.9	1.2
NRRE α -4-C5-IR	grgfyqvflllqtea	18.0	23.3	26.9	0.9	1.2
NRRE α -4-C6-IR	qongfyrifdtilldwv	7.8	13.6	15.7	0.9	1.2
NRRE α -4-C12-IR	fagsfyrfeqlllsdq	12.3	16.7	19.9	0.8	1.2
NRRE α -4-D7-IR	dpnafyrfeegllwreh	10.2	23.7	27.9	0.8	1.2
NRRE α -4-D11-IR	?glnfyrfvglltdtl	5.4	19.3	22.3	0.9	1.2
NRRE β -4-F1-IR	rhinfygyfddllatwh	21.7	23.0	28.6	0.8	1.2
NRRE β -4-F9-IR	fhrgfyrfeinllsgda	10.1	18.4	22.5	0.8	1.2
NRRE β -4-F12-IR	mgssfyrfettllggl	4.5	13.5	16.6	0.8	1.2
NRRE β -4-A3-IR	gsldfysfmerllglp	16.4	22.3	26.8	0.8	1.2
NRRE α -1-A7-IR	stvsfyrfyallqspc	16.9	1.2	1.3	0.9	1.1
NRRE α -4-C11-IR	lgyfyfryfedllnhos	7.8	19.7	21.2	0.9	1.1
NRRE α -4-D8-IR	dhrgfyrfyqlagnv	6.9	17.6	20.1	0.9	1.1
NRRE α -4-D10-IR	eysgfygfynhllgslg	6.4	17.2	19.5	0.9	1.1
NRRE α -4-E5-IR	tsnwfyqvfddllaged	13.2	26.1	27.6	0.9	1.1
NRRE α -4-E8-IR	ssgfyfryfsqlltemn	8.7	22.9	24.2	0.9	1.1
NRRE α -4-E10-IR	vhgefyrifefllretf	3.5	12.4	13.2	0.9	1.1
NRRE β -4-F8-IR	sdsgefyrfaqllygvt	8.1	22.9	25.2	0.9	1.1
NRRE β -4-F10-IR	etggfygyfqaillatyh	5.3	17.9	19.1	0.9	1.1
NRRE β -4-C8-IR	gdrgfyrfemllndfg	10.6	27.2	28.9	0.9	1.1
NRRE β -4-H3-IR	fggafyrfeallgemg	3.9	24.2	25.7	0.9	1.1
NRRE β -4-H9-IR	dggafyrfeallgeld	4.1	26.5	29.3	0.9	1.1
NRRE β -4-H10-IR	whsdfyrfellsllqedg	3.4	22.3	24.6	0.9	1.1
NRRE γ -4-A6-IR	beegfygyfrrllgver	14.9	25.8	27.6	0.9	1.1
NRRE γ -4-A8-IR	mdagfygyfddllanwg	9.8	22.8	24.7	0.9	1.1

FIGURE 2P (Con't)

Clone Design	Sequence XXXXXXXXXX	E-Tag	ICFR	IR	ICFR/IR	IR/ICFR
NNRP-4-A10-IR	SGFAFYQFQELLAGHD	7.6	20.3	22.0	0.9	1.1
NNRP-4-B6-IR	GDGFYGYFASILLSGEG	12.2	22.3	24.2	0.9	1.1
NNRP-4-B9-IR	EANGFYRYFDILLDFG	6.7	22.9	25.9	0.9	1.1
NNRP-4-C8-IR	AVNGFYRYFNRLLEDE	8.5	16.3	16.0	1.0	1.0
NNRP-4-C9-IR	QDGFYRYFDILLDEVA	5.6	20.7	19.9	1.0	1.0
NNRP-4-C10-IR	ISQGFYGYFSRLQDTE	6.7	16.5	17.2	1.0	1.0
NNRP-4-E11-IR	YSTGFYRYFDILLDQMP	6.0	20.3	20.9	1.0	1.0
NNRP-4-F11-IR	PNQGFYRYFDILLGSG	7.7	21.8	21.9	1.0	1.0
NNRP-4-G2-IR	RHQAFYSYFRDLPRECP	19.1	24.7	25.6	1.0	1.0
NNRP-4-G9-IR	ETGPFYRYFEELLQAQA	7.8	27.3	26.4	1.0	1.0
NNRP-4-H7-IR	AGDRFYDYFORILLADYD	2.6	26.6	27.9	1.0	1.0
NNRP-4-H8-IR	GGSGFYRYFMGLLAQE	3.6	23.0	24.1	1.0	1.0
NNRP-4-B1-IR	LLNRLRYFAGAGWFG	17.6	24.5	23.4	1.0	1.0
NNRP-4-B10-IR	DGSGFYRYFEMLLGSL	5.5	18.3	19.0	1.0	1.0
NNRP-1-B3-IR	RDMAFYRYFSLLESFQ	16.4	13.4	12.7	1.1	0.9
NNRP-2-C2-IR	GNAGFYRISRLMQSTE	22.5	24.4	21.3	1.1	0.9
NNRP-2-C3-IR	GNAGFYRYFADLMAGYE	19.6	21.7	19.7	1.1	0.9
NNRP-2-D10-IR	YQAFYRYFATLLSTTD	17.8	6.3	5.4	1.2	0.9
NNRP-3-E11-IR	GGGFYRYFQILLGSGG	12.9	10.8	9.6	1.1	0.9
NNRP-3-F5-IR	DGSGFYGYFDVLRQFE	25.1	18.3	17.0	1.1	0.9
NNRP-3-F8-IR	VGSGFYRYFDQILLMYG	22.2	15.7	13.9	1.1	0.9
NNRP-3-F10-IR	YGTDFYLYFDQILLQYV	20.5	14.6	13.1	1.1	0.9
NNRP-3-G7-IR	FNSSFYLYFDRLNTVG	21.0	18.3	15.6	1.2	0.9
NNRP-4-C2-IR	RAAGFYRYFEDLLGARG	25.5	25.1	23.3	1.1	0.9
NNRP-4-D12-IR	TGAGFYRYFDILLGTG	14.7	19.7	18.5	1.1	0.9
NNRP-4-G3-IR	RLEFYGYFOELLRLNF	14.6	27.8	25.7	1.1	0.9
NNRP-4-G4-IR	GMGPFYRYFDILLRESD	20.0	28.6	24.9	1.1	0.9
NNRP-4-A5-IR	HGDGFYQYFMEVIRLQN	17.0	29.0	27.3	1.1	0.9

FIGURE 2P (Con't)

Clone Design	Sequence XXXXXXXXFYRFYXLLXXXXXX	E-Tag	IGFR	IR	IGFR/IR	IR/IGFR
NNR γ -4-A12-IR	AFYRFYRLLFSFG	4.9	16.3	14.9	1.1	0.9
NNR γ -4-B8-IR	DRGFYRFESLLGSS	6.1	21.3	19.9	1.1	0.9
NNR α -1-A5-IR	LSTSFYQVIAGLLRGDR	2.3	1.4	1.1	1.2	0.8
NNR α -1-B7-IR	GSSGFYRFNNMLSGT	19.2	15.7	12.4	1.3	0.8
NNR α -2-C7-IR	DRGFYRFEGLLASVG	19.6	20.0	16.5	1.2	0.8
NNR α -2-C11-IR	NSAAFYRFQELLEVEV	20.1	20.0	16.3	1.2	0.8
NNR α -2-C12-IR	LSDFYRFQLLMGARS	14.3	10.1	8.5	1.2	0.8
NNR α -2-D12-IR	RSTLFYRFQILLEVG	11.5	11.4	9.3	1.2	0.8
NNR α -3-G2-IR	TRGGFYRFEDLLQVYS	20.8	20.7	16.1	1.3	0.8
NNR α -3-G8-IR	GVSGFYRFQSLDSVG	14.7	11.0	9.2	1.2	0.8
NNR α -3-G10-IR	QNDAFYSFNSLLQAYT	18.8	16.5	13.9	1.2	0.8
NNR α -3-G11-IR	RQDFYRFQLLLEEV	12.0	10.3	8.5	1.2	0.8
NNR α -3-G12-IR	EGSGFYRFKILLQSP	11.7	11.8	9.3	1.3	0.8
NNR γ -4-B2-IR	RHKAIFYRFEELOKNV	22.8	30.3	25.3	1.2	0.8
NNR α -1-B8-IR	GRWTRLIVRSTVISRELLHYSL	16.1	10.1	6.9	1.5	0.7
NNR α -2-C5-IR	QALSFYRFERLLDEVS	18.1	19.2	13.7	1.4	0.7
NNR α -2-C9-IR	SKSAFYRFDELLGNSG	22.9	21.7	16.1	1.3	0.7
NNR α -2-D2-IR	LGGAIFYRFQLLNSHV	26.1	26.2	17.6	1.5	0.7
NNR α -2-D5-IR	INSGFYGYFQLLSGHQ	21.7	21.1	15.4	1.4	0.7
NNR α -2-D11-IR	SQSSFYRFESLLEONP	12.3	10.8	7.8	1.4	0.7
NNR α -3-E2-IR	ADGGFYGYFAALLGSVS	24.4	25.5	18.3	1.4	0.7
NNR α -3-E4-IR	QNGSFYRFYFALLGDSG	23.0	22.3	14.7	1.5	0.7
NNR α -3-F4-IR	WDTGFYRFYFELLEDRD	24.9	25.1	17.6	1.4	0.7
NNR α -3-G4-IR	HPRDFYRFERLLNQVD	20.9	20.4	14.1	1.5	0.7
NNR α -3-H4-IR	DGGAIFYRFMDLLGAHE	17.7	17.6	11.6	1.5	0.7
NNR α -4-E12-IR	AGRGFYRFEFHLLAGRE	4.3	15.4	10.8	1.4	0.7
NNR β -4-G11-IR	SSRGFYRFYFELLEADSW	6.6	18.4	13.1	1.4	0.7
NNR β -4-H6-IR	KYSGFYEFYFALLGRRE	2.2	16.1	11.7	1.4	0.7

FIGURE 2P (Con't)

Clone Design	Sequence	E-Tag	IGFR	IR	IGFR/IR	IR/IGFR
NRPF-4-H11-IR	XXXXXXXXFYFXXLLXXXXX	--	--	--	--	--
NRPF-4-H11-IR	DYAFYGYNNLLTSG	2.3	12.4	9.0	1.4	0.7
NRPF-1-B1-IR	FQSSFYGYFESLMSYK	18.8	18.7	11.5	1.6	0.6
NRPF-2-D7-IR	DTNAFYFEGLLMSEH	21.0	21.8	13.2	1.6	0.6
NRPF-2-D8-IR	GSSFYFYFEQLAQWE	20.2	19.8	12.2	1.6	0.6
NRPF-3-E1-IR	SQGGFYFYFEKLDEVT	20.0	20.5	12.9	1.6	0.6
NRPF-3-E5-IR	RSGLFYFYFEQLQAI	20.0	24.5	15.5	1.6	0.6
NRPF-3-H3-IR	QGGFYFYFLSLEEVG	19.8	19.1	12.2	1.6	0.6
NRPF-3-H5-IR	WRGAFYFYFTLLSDEG	19.9	18.0	11.1	1.6	0.6
NRPF-1-A1-IR	AAQFYFYSLGSDQT	24.4	14.9	7.9	1.9	0.5
NRPF-3-E6-IR	RNSGFYFYQHLVSEWE	23.1	19.0	9.6	2.0	0.5
NRPF-3-F9-IR	QHLEFYFYFAELGRDT	21.1	18.8	9.6	1.9	0.5
NRPF-3-G6-IR	QIDFYFYFADLRGFA	22.4	17.7	9.0	2.0	0.5
NRPF-3-H9-IR	LGGFYFYFNLVWMSG	18.3	13.1	6.9	1.9	0.5
NRPF-1-A8-IR	GDRAFYFYQRLGEGW	16.9	13.8	5.7	2.4	0.4
NRPF-1-A9-IR	CEDAFYFYFVNLQGC	16.5	15.2	5.6	2.7	0.4
NRPF-2-D6-IR	NYQFYFYFEMLEGEV	19.4	18.5	6.8	2.7	0.4
NRPF-3-F6-IR	VGDAFYFYQGLLRQDQ	22.8	19.5	7.9	2.5	0.4
NRPF-3-H2-IR	MHGSFYFYQDLQAPP	19.9	18.9	8.5	2.2	0.4
NRPF-1-B6-IR	DVGDYFYFGLLLTSDR	14.1	11.5	3.9	3.0	0.3
NRPF-2-C6-IR	NSAIFYFYFSQLAQIR	18.4	19.2	4.1	4.7	0.2
NRPF-4-A1-IR	IIGFSYFYFNSVLRLGT	9.7	10.9	1.8	6.0	0.2
NRPF-4-A7-IR	RDPFYFYFVNLGASA	2.5	6.3	1.3	4.9	0.2
NRPF-4-A9-IR	EGSGFYFYFSLGLOG	3.0	10.0	1.4	7.1	0.1
NRPF-4-B11-IR	LKQGYFYFWQLHLGS	4.1	18.7	1.2	15.5	0.1

FIGURE 2P (Cont')

Clone	Sequence	Ratios over Background		Comparisons	
Design		E-Tag	IR	IGFR/IR	IR/IGFR
R20-4-F9-IGFR	XXXXXXXXXXXXXXXXXXXX	--	--	--	--
R20-4-H4-IGFR	PLAELWAYFEHSEQRSSAH	33.1	19.3	1.0	19.3 0.1
R20-4-F9-IGFR	PVLSGLLYFAGGPLQPOQS	24.1	5.6	3.2	1.8 0.6
R20-4-D6-IGFR	GGYLDLWHYFRDGAIQPW	2.5	2.4	1.4	1.7 0.6
R20-4-G2-IGFR	VDQRQCGWLLALENYFRSTV	6.1	2.9	1.9	1.5 0.7
	DVPAGGLLRQMWVYFRSDP	6.3	2.2	2.0	1.1 0.9

Figure 3A

000200-00000000

Clone Design	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGF5R	IR	IGFR/IR	IR/IGFR
20C-3-F3-IGFR	XXXXXXXXXXXXXXXXXXXX	--	--	--	--	--
	RRVACTQADGLLCESDPLKALLSYF	35.5	32.8	17.9	1.8	0.5

Figure 3B

Clone	Design	Sequence	Ratios over Background			Comparisons		
			E-Tag	IGF5R	IR	IGF5R/IR	IR/IGF5R	
RB6-4-B10-IGFR	RB6-4-B10-IGFR	XXXLXXLXXYFXXXXX	33.1	26.9	1.0	26.9	<0.1	
RB6-3-E6-IGFR	RB6-3-E6-IGFR	RPVLGWLDFYFASDPM	37.6	26.6	1.0	26.6	<0.1	
RB6-4-B9-IGFR	RB6-4-B9-IGFR	RWPLSALMDYFRSDGV	39.2	26.3	1.0	26.3	<0.1	
RB6-3-F5-IGFR	RB6-3-F5-IGFR	DGVLASLMRYFSSGTL	33.2	15.6	1.0	15.6	0.1	
RB6-3-B6-IGFR	RB6-3-B6-IGFR	DRQLGWLMDYFHLTLP	37.4	13.3	1.0	13.3	0.1	
RB6-3-D4-IGFR	RB6-3-D4-IGFR	DGILGLMAYFVS7RV	31.2	20.7	2.0	10.4	0.1	
RB6-4-D10-IGFR	RB6-4-D10-IGFR	QDILLGLMLYFAETDV	31.7	10.1	1.0	10.1	0.1	
RB6-3-D6-IGFR	RB6-3-D6-IGFR	SGVLADLRFYFQHPWP	33.9	9.9	1.0	9.9	0.1	
RB6-4-F9-IGFR	RB6-4-F9-IGFR	DPPLGLMTYFSSDPG	34.3	28.3	3.0	9.4	0.1	
RB6-3-E1-IGFR	RB6-3-E1-IGFR	DSVLRSLYSFASGDIA	30.5	9.3	1.0	9.3	0.1	
RB6-3-B2-IGFR	RB6-3-B2-IGFR	DGVLAALEAYFRGPRD	22.2	8.8	1.0	8.8	0.1	
RB6-3-D7-IGFR	RB6-3-D7-IGFR	DEILGALYSYFSLSGA	31.2	7.6	1.0	7.6	0.1	
RB6-4-C11-IGFR	RB6-4-C11-IGFR	QDVLGALQRYFASGEPW	33.7	7.6	1.0	7.6	0.1	
RB6-4-F12-IGFR	RB6-4-F12-IGFR	DSVLQYLLNHFGDSKQ	38.1	7.3	1.0	7.3	0.1	
RB6-4-F7-IGFR	RB6-4-F7-IGFR	NEVLEGLSFYFV?ANG	38.6	7.3	1.0	7.3	0.1	
RB6-3-G6-IGFR	RB6-3-G6-IGFR	SGILGQLLRYFKGAGG	34.0	7.1	1.0	7.1	0.1	
RB6-4-E9-IGFR	RB6-4-E9-IGFR	DELLDLMAQYFQGGDL	37.6	6.9	1.0	6.9	0.1	
RB6-4-G10-IGFR	RB6-4-G10-IGFR	PGILLDLWRYPASPDQ	34.9	14.5	2.2	6.6	0.2	
RB6-4-B12-IGFR	RB6-4-B12-IGFR	DSVLLDLYEFSSGSSG	36.3	28.9	4.5	6.4	0.2	
RB6-3-B5-IGFR	RB6-3-B5-IGFR	DGMLSLRLMEYFATNVP	17.2	6.1	1.0	6.1	0.2	
RB6-3-C5-IGFR	RB6-3-C5-IGFR	DVILGGLMDYFASGGH	38.7	15.8	2.9	5.4	0.2	
RB6-4-B8-IGFR	RB6-4-B8-IGFR	GGVLAALERYFRYSAGD	31.9	22.0	4.1	5.4	0.2	
RB6-3-H2-IGFR	RB6-3-H2-IGFR	DEVILGLRMAYFAGESLG	31.8	22.4	4.0	5.3	0.2	
RB6-3-E5-IGFR	RB6-3-E5-IGFR	DGILQLSLMDYFARSPVG	37.0	20.5	4.0	5.1	0.2	
RB6-3-B3-IGFR	RB6-3-B3-IGFR	VDILSELMDYFRGEGG	31.7	4.8	1.0	4.8	0.2	
RB6-4-G7-IGFR	RB6-4-G7-IGFR	DKVIRLLGLEYFATHKSG	37.4	10.0	2.1	4.8	0.2	
RB6-3-A3-IGFR	RB6-3-A3-IGFR	QGPLAWLRDVFASGTRS	27.2	4.7	1.0	4.7	0.2	
RB6-4-E9-IGFR	RB6-4-E9-IGFR	QDVIRLSLLSYFMNGNDV	37.3	20.1	4.8	4.2	0.2	

Figure 3D

Clone	Design	Sequence	Ratios over Background			Comparisons		
			E-Tag	IGF5R	IR	IGFR/IR	IR/IGFR	--
YB6-3-F1-IGFR	XXLXXLXXYFXXXXX	NTILGDLWRYFAGSGM	26.5	5.8	1.4	4.1	0.2	--
YB6-4-B7-IGFR	2DVLKKLVYFELSGA	GGPLQGLTYFKQSPVC	31.1	11.4	2.9	3.9	0.3	--
YB6-4-C10-IGFR	GGPLQGLTYFKQSPVC	DRLLSGLWAYFAGNGGS	32.2	3.7	1.0	3.7	0.3	--
YB6-3-A1-IGFR	DLILQSLLDYFQRPVG	25.1	21.1	3.5	1.0	3.5	0.3	--
YB6-3-F6-IGFR	LALLPMLMDYFVATDQ	35.5	35.5	18.1	5.6	3.2	0.3	--
YB6-3-H5-IGFR	DSLRLRLDVFARHIA	36.2	36.2	22.5	7.5	3.0	0.3	--
YB6-4-D8-IGFR	DGVLGQLWYFQAQYFGS	41.1	41.1	30.6	10.6	2.9	0.3	--
YB6-4-A8-IGFR	?PPDLRALMEYFTGARD	38.7	33.0	11.5	2.9	0.3	--	--
YB6-4-H8-IGFR	DNVLEGLMSYFALWSQL	20.9	2.2	1.0	2.2	0.5	--	--
YB6-3-E2-IGFR	SAVLEYLLAYFARTGAA	31.0	2.1	1.0	2.1	0.5	--	--
YB6-3-C2-IGFR	DRALGPLARVFMVNNQ	38.7	5.5	2.6	2.1	0.5	--	--
YB6-4-G8-IGFR	WRILDLRLAYFKESQGD	32.8	2.0	1.0	2.0	0.5	--	--
YB6-3-G5-IGFR	DDVLVTLFQYFRASGTG	37.6	30.2	15.1	2.0	0.5	--	--
YB6-4-C9-IGFR	FDVLTWLGRYF*WNTGK	36.6	5.5	3.0	1.8	0.5	--	--
YB6-4-D11-IGFR	RDVLGDLREYFRASVGG	25.2	4.2	2.4	1.8	0.6	--	--
YB6-4-B11-IGFR	IKTLNDLLAYFRGDLGV	38.1	29.8	22.2	1.3	0.7	--	--
YB6-3-G3-IGFR	DEALLMLWRYFRGSPSP	31.6	8.7	7.2	1.2	0.8	--	--
YB6-4-H12-IGFR	ESPDRLALRAYFVSGRNW	40.1	2.8	2.5	1.1	0.9	--	--
YB6-4-G12-IGFR	IQSL*DLIQYFVSPSPV	36.7	32.5	31.4	1.0	1.0	--	--
YB6-3-C4-IGFR	GGILDT?LDYFRSTDVG	37.1	6.2	13.5	0.5	2.2	--	--

Figure 3D (Con't)

Clone	Design	Sequence	Ratios over Background			Comparisons		
			E-Tag	IGFαR	IR	IGFβR	IR/IGFβR	
P815-4-H9-IR		HLCVLEELFWGASLFGQCSG	34.9	0.9	37.6	<0.1	<0.1	40.8
P815-3-B1-IR		PLCVLEELFWNSTLFGQCSY	31.7	0.9	35.8	<0.1	<0.1	39.3
P815-3-D1-IR		HLCVLEELFWGASLFGQCSG	30.4	0.9	33.5	<0.1	<0.1	38.9
P815-3-D4-IR		PLCVLEELFWGASLFGQCSG	31.5	0.9	33.6	<0.1	<0.1	38.8
P815-3-CS-IR		HLCVLEELFWGASLFGQCSG	31.1	0.8	31.2	<0.1	<0.1	38.5
P815-4-H3-IR		NLCVLEELFWGASLFGQCSG	33.7	1.0	37.2	<0.1	<0.1	38.4
P815-3-A5-IR		PLCVLEELFWGASLFGQCSG	37.4	1.1	40.9	<0.1	<0.1	38.3
P815-3-D7-IR		QLCVLEELFWGASLFGQCSG	33.6	0.9	34.3	<0.1	<0.1	38.3
P815-3-A1-IR		HLCVLEELFWGASLFGQCSG	29.8	0.9	34.8	<0.1	<0.1	38.0
P815-4-H4-IR		PLCVLEELFWGASLFGQCSG	31.1	0.9	32.7	<0.1	<0.1	38.0
P815-3-A3-IR		HLCVLEELFWGASLFGQCSG	32.8	1.0	39.1	<0.1	<0.1	37.9
P815-3-B3-IR		KLCVLEELFWGASLFGQCSG	33.7	1.0	37.5	<0.1	<0.1	37.5
P815-3-A4-IR		YLCVLEELFWGASLFGQCSG	32.5	1.0	36.9	<0.1	<0.1	37.5
P815-3-D2-IR		HLCVLEELFWGASLFGQCSG	31.9	0.9	34.1	<0.1	<0.1	37.4
P815-3-C4-IR		QLCVLEELFWGASLFGQCSG	31.6	0.8	31.8	<0.1	<0.1	37.4
P815-3-B4-IR		HLCVLEELFWGASLFGQCSG	33.8	1.0	36.7	<0.1	<0.1	37.3
P815-3-C1-IR		HLCVLEELFWGASLFGQCSG	29.0	0.9	35.0	<0.1	<0.1	37.3
P815-4-G9-IR		SLCVLEELFWGASLFGQCSG	36.5	1.0	38.9	<0.1	<0.1	37.1
P815-4-G6-IR		HLCVLEELFWGASLFGQCSG	34.9	1.0	36.4	<0.1	<0.1	37.0
P815-3-A8-IR		QLCVLEELFWGASLFGQCSG	34.7	1.1	39.3	<0.1	<0.1	36.9
P815-4-G5-IR		PLCVLEELFWGASLFGQCSG	26.5	1.0	35.1	<0.1	<0.1	36.8
P815-3-B9-IR		HLCVLEELFWGASLFGQCSG	33.2	0.9	34.1	<0.1	<0.1	36.8
P815-4-F4-IR		PLCVLEELFWGASLFGQCSG	28.6	0.8	30.0	<0.1	<0.1	36.7
P815-3-A2-IR		QLCVLEELFWGASLFGQCSG	32.5	1.0	36.6	<0.1	<0.1	36.6
P815-3-B6-IR		HLCVLEELFWGASLFGQCSG	31.6	0.9	32.9	<0.1	<0.1	36.5
P815-4-H7-IR		DLCVLEELFWGASLFGQCSG	33.7	1.0	37.6	<0.1	<0.1	36.4
P815-4-H8-IR		QLCVLEELFWGASLFGQCSG	35.8	1.0	37.0	<0.1	<0.1	36.4
P815-4-G7-IR		NLCVLEELFWGASLFGQCSG	33.7	1.0	35.8	<0.1	<0.1	36.3

Figure 4B

Clone Design	Sequence	Ratios over Background				Comparisons	
		E-Tag	IR	IGF5R	IR/IGF5R	IGF5R/IR	IR/IGF5R
F815-3-A6-IR	HLCVLEELFMGASLFGQCSG	34.6	1.1	39.0	<0.1	<0.1	36.2
F815-3-D3-IR	QLCVLEELFMGASLFGQCSG	33.8	1.0	36.2	<0.1	<0.1	36.2
F815-3-B12-IR	DLCVLEELFMGASLFGQCSG	33.2	1.0	35.7	<0.1	<0.1	36.2
F815-4-G10-IR	YLCVLEELFMGASLFGQCSG	35.4	1.0	37.2	<0.1	<0.1	36.1
F815-4-E3-IR	HLCVLEELFMGASLFGQCSG	32.4	1.0	35.0	<0.1	<0.1	36.1
F815-4-E6-IR	PLCVLEELFMGASLFGQCSG	33.2	1.0	34.5	<0.1	<0.1	36.1
F815-4-F1-IR	HLCVLEELFMGASLFGQCSG	29.4	0.9	32.5	<0.1	<0.1	36.0
F815-4-G8-IR	PLCALEELFMGASLFGQCSG	36.8	1.1	38.2	<0.1	<0.1	35.9
F815-4-H12-IR	HLCVLEELFMGASLFGQCSG	30.5	0.9	31.9	<0.1	<0.1	35.9
F815-4-G3-IR	PLCVLEELFMGASLFGQCSG	31.4	1.0	35.7	<0.1	<0.1	35.7
F815-3-C2-IR	DLGLLEELFMGASLFGQCSG	32.3	1.0	36.1	<0.1	<0.1	35.6
F815-4-E10-IR	HLCVLEELFMGASLFGQCSG	35.4	1.0	36.5	<0.1	<0.1	35.4
F815-3-A12-IR	HLCVLEELFMGASLFGQCSG	32.1	1.0	36.3	<0.1	<0.1	35.3
F815-3-B8-IR	HLCVLEELFMGASLFGQCSG	33.6	1.0	35.8	<0.1	<0.1	35.3
F815-3-B2-IR	HLCVLEELFMGASLFGQCSG	31.0	1.0	35.3	<0.1	<0.1	35.3
F815-3-C3-IR	PLCVLEELFMGASLFGQCSG	30.1	1.0	35.3	<0.1	<0.1	35.3
F815-3-A7-IR	HLCVLEELFMGASLFGQCSG	33.1	1.0	35.8	<0.1	<0.1	35.2
F815-4-F9-IR	RLCVLEELFMGASLFGQCSG	33.4	1.0	35.7	<0.1	<0.1	35.2
F815-3-B7-IR	QLCVLEELFMGASLFGQCSG	32.0	1.0	33.5	<0.1	<0.1	35.0
F815-4-E4-IR	HLCVLEELFMGASLFGQCSG	28.0	1.0	33.4	<0.1	<0.1	35.0
F815-4-E12-IR	YLCVLEELFMGASLFGQCSG	28.0	0.9	30.2	<0.1	<0.1	34.8
F815-4-F8-IR	HLCVLEELFMGASLFGQCSG	33.8	1.0	35.2	<0.1	<0.1	34.7
F815-3-C7-IR	HLCVLEELFMGASLFGQCSG	33.9	1.0	34.7	<0.1	<0.1	34.7
F815-4-F10-IR	PLCVLEELFMGASLFGQCSG	32.7	1.0	34.2	<0.1	<0.1	34.7
F815-3-D11-IR	HLCVLEELFMGASLFGQCSG	35.4	1.1	37.3	<0.1	<0.1	34.6
F815-4-E7-IR	HLCVLEELFMGASLFGQCSG	30.3	0.9	32.2	<0.1	<0.1	34.6
F815-3-A10-IR	QLCVLEELFMGASLFGQCSG	34.0	1.1	36.4	<0.1	<0.1	34.3
F815-3-B11-IR	ALCVLEELFMGASLFGQCSG	33.7	1.1	36.3	<0.1	<0.1	34.2

Figure 4B (Con't)

Clone Design	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGfsR	IR	IGR/IR	IR/IGR	
F815-4-F11-IR	HLCVLEELFWGASLFGQCSG	--	--	--	--	--	--
F815-3-A9-IR	RLCVLEERFWGASLFGQCSG	31.8	1.0	33.7	<0.1	34.2	<0.1
F815-4-G11-IR	PLCVLEELFWGASLFGQCSG	31.9	1.0	35.5	<0.1	34.1	<0.1
F815-3-D8-IR	SLCVLEELFWGASLFGQCSG	32.3	1.0	34.4	<0.1	33.9	<0.1
F815-4-G4-IR	HLCVLEEFWASLFGQCSG	32.3	1.0	33.3	<0.1	33.7	<0.1
F815-3-C8-IR	DLCLLEELLWASLFGQCSG	23.8	1.0	32.2	<0.1	33.7	<0.1
F815-4-G12-IR	YLCVLEERFWGASLFGQCSG	33.9	1.0	35.1	<0.1	33.6	<0.1
F815-3-D12-IR	QLCVLEEFWASLFGQCSG	31.7	1.0	33.5	<0.1	33.5	<0.1
F815-4-F7-IR	QLCVLEELWASLFGQCSG	33.3	1.0	34.8	<0.1	33.4	<0.1
F815-4-F2-IR	HLCVLEELFWGASLFGQCSG	26.1	1.0	34.3	<0.1	33.4	<0.1
F815-3-B9-IR	HLCVLEELFWGASLFGQCSG	33.6	1.1	33.8	<0.1	33.3	<0.1
F815-4-H2-IR	PLCVLEELFWGASLFGQCSG	36.1	1.2	35.7	<0.1	33.2	<0.1
F815-4-E11-IR	HLCVLEELFWGASLFGQCSG	33.2	1.1	38.4	<0.1	33.0	<0.1
F815-4-G1-IR	QLCVLEELWASLFGQCSG	27.9	1.0	35.4	<0.1	33.0	<0.1
F815-3-A11-IR	HLCVLEELFWGASLFGQCSG	37.7	1.2	31.5	<0.1	32.8	<0.1
F815-4-F6-IR	HLCVLEELWASLFGQCSG	32.3	1.1	40.1	<0.1	32.7	<0.1
F815-3-D9-IR	RLCVLEELWASLFGQCSG	31.4	1.0	34.6	<0.1	32.6	<0.1
F815-3-C11-IR	RLCVLEELWASLFGQCSG	33.4	1.1	32.5	<0.1	32.5	<0.1
F815-4-G2-IR	HLCVLEELFWGATLFGQCSG	30.2	1.1	35.7	<0.1	31.9	<0.1
F815-3-C9-IR	HLCVLEELFWGASLFGQCSG	29.7	1.0	34.3	<0.1	31.4	<0.1
F815-4-H10-IR	HLCVLEELFWASLFGQCSG	31.9	0.9	31.4	<0.1	31.0	<0.1
F815-4-F3-IR	HLCVLEELWASLFGQCSA	19.4	1.0	27.6	<0.1	29.4	<0.1
F815-4-F5-IR	RLCVLEELFWASLFGQCSG	12.3	0.9	28.0	<0.1	28.9	<0.1
F815-4-H1-IR	PLCVLEELFWASLFGQCSG	6.9	1.0	24.8	<0.1	26.8	<0.1
F815-4-E5-IR	PLCVLEELFWASLFGQCSG	3.5	1.0	15.8	<0.1	16.5	<0.1
F815-4-H5-IR	PLCVLEELFWASLFGQCSG	5.5	1.0	13.6	<0.1	14.0	<0.1
F815-3-C10-IR	QLCVLG#RFGWGSGLGVCSD	3.5	1.1	13.1	<0.1	13.5	<0.1
				5.2		0.2	

Figure 4B (Con't)

Clone	Parental/Design	Sequence	Ratios over Background			Comparisons	
			E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
F820-4-B5-IR		HLCLLEELFWGASLFGYCSG	39.1	1.8	27.7	0.1	15.4
F820-4-A2-IR		HLCLLEELFWGASLFGYCSG	28.1	0.9	17.9	<0.1	21.1
F820-4-E2-IR		HLCLLEELFWGASLFGYCSG	34.0	1.6	22.7	0.1	13.9
F820-4-D10-IR		TLCAFWNGSGVRCSVAVV	21.3	0.7	9.0	0.1	13.4
F820-4-H7-IR		PLCGLKN.SGVRLCSPALV	34.1	1.0	12.1	0.1	12.1
F820-4-G6-IR		PLCLQBELFWGASLFGYCSG	14.2	0.6	6.5	0.1	11.6
F820-4-C2-IR		DLCLLEELFWGASLFGYCSG	14.0	0.5	6.1	0.1	11.5
F820-4-B4-IR		PLCLLEELFWGASLFGYCSG	38.1	1.2	11.8	0.1	9.9
F820-4-C7-IR		PLCLLEELFWGASLFGYCSG	15.1	0.7	6.4	0.1	8.7
F820-4-F10-IR		PLCLLEELFWGASLFGYCSG	46.3	2.7	22.2	0.1	8.2
F820-4-G5-IR		GLCLLEELFWGASLFGYCSG	14.5	0.6	4.7	0.1	8.0
F820-4-F2-IR		PLCWLLEELFWGASLFGYCSG	8.8	0.6	4.4	0.1	7.5
F820-4-H8-IR		RLCLLEELFWGASLFGYCSG	11.7	0.6	4.2	0.1	7.4
F820-4-D7-IR		PLCLLEELFWGASLFGYCSG	16.0	0.6	4.7	0.1	7.3
F820-4-B2-IR		NLCLLEELFWGASLFGYCSG	14.5	0.8	5.9	0.1	7.1
F820-4-C3-IR		QLCLLEELFWGASLFGYCSG	5.0	0.4	2.4	0.2	6.9
F820-4-H4-IR		HLCLLEELFWGASLFGYCSG	37.5	1.1	7.5	0.2	6.6
F820-4-B10-IR		PLCLLEELFWGASLFGYCSG	21.2	1.1	6.4	0.2	5.9
F820-4-A5-IR		PLCLLEELFWGASLFGYCSG	7.5	0.7	3.7	0.2	5.6
F820-4-F6-IR		QLCLLEELFWGASLFGYCSG	5.3	0.8	4.4	0.2	5.2
F820-4-F1-IR		PLCLLEELFWGASLFGYCSG	5.7	0.6	3.0	0.2	4.9
F820-4-A3-IR		HLCLLEELFWGASLFGYCSG	9.1	1.0	4.7	0.2	4.7
F820-4-D1-IR		DLCLLEELFWGASLFGYCSG	5.9	0.8	3.5	0.2	4.5
F820-4-F5-IR		DLCLLEELFWGASLFGYCSG	25.7	2.3	10.5	0.2	4.5
F820-4-F12-IR		OLCLLEELFWGASLFGYCSG	3.7	0.6	2.7	0.2	4.2
F820-4-A11-IR		HLCLLEELFWGASLFGYCSG	11.3	0.6	2.2	0.3	3.5
F820-4-E8-IR		PLCLLEELFWGASLFGYCSG	7.6	0.8	2.7	0.3	3.5
F820-4-H3-IR		HLCLLEELFWGASLFGYCSG	8.0	1.2	4.3	0.3	3.5
			17.5	2.6	9.0	0.3	3.4

Figure 4D

Clone	Parental/Design	Sequence	Ratios over Background		Comparisons	
			E-Tag	IGFsR	IGFR/IR	IR/IGFR
F820-4-A8-IR		HLCVLEELFWGASLFGYCSG	6.4	0.7	2.4	0.3 / 3.4
F820-4-A8-IR		QLCVMEELFWGASLFGYCSG	3.9	0.6	1.9	0.3 / 3.4
F820-4-G1-IR		HLCVLEELFWGASLFGYCSG	9.8	1.3	3.6	0.4 / 2.9
F820-4-F3-IR		QLCVLEELFWGASLFGYCSG	5.4	1.2	3.2	0.4 / 2.6
F820-4-D6-IR		PLCVLEELFWGASLFGYCSG	25.5	2.4	6.1	0.4 / 2.5
F820-4-A1-IR		YLCVQELFWGASLFGYCSG	15.9	1.6	4.1	0.4 / 2.5
F820-4-H2-IR		HLCVLEELFWGASLFGYCSG	6.8	1.9	4.7	0.4 / 2.5
F820-4-F4-IR		HLCVLEELFWGASLFGYCSG	4.1	0.8	1.9	0.4 / 2.4
F820-4-B6-IR		QLCVLEELFWGASLFGYCSG	22.2	3.1	7.0	0.4 / 2.3
F820-4-B11-IR		HLCVLEELFWGASLFGYCSG	4.1	1.1	2.4	0.5 / 2.2
F820-4-H6-IR		QLCVLEELFWGASLFGYCSG	3.1	0.9	1.9	0.5 / 2.1
F820-4-H9-IR		PLCVLEELFWGASLFGYCSG	4.6	1.3	2.5	0.5 / 1.9
F820-4-D3-IR		QLCVLEELFWGASLFGYCSG	13.0	1.1	2.1	0.5 / 1.9
F820-4-C1-IR		QLCVLEELFWGASLFGYCSG	10.4	1.2	2.0	0.6 / 1.7
F820-4-D12-IR		QLCVLEELFWGASLFGYCSG	7.2	2.2	3.4	0.6 / 1.5
F820-4-B8-IR		QLCVLEELFWGASLFGYCSG	13.9	2.5	2.8	0.9 / 1.1
F820-4-C6-IR		HLCVLEELFWGASLFGYCSG	5.3	2.6	2.9	0.9 / 1.1
F820-4-C10-IR		HLCVLEELFWGASLFGYCSG	3.5	2.3	2.1	1.1 / 0.9
F820-4-D4-IR		QLCVLEELFWGASLFGYCSG	1.6	1.6	1.5	1.1 / 0.9
F820-4-E1-IR		HLCVLEELFWGASLFGYCSG	15.9	0.6	5.0	1.2 / 0.8
F820-4-B3-IR		HLCVLEELFWGASLFGYCSG	7.8	3.2	2.5	1.3 / 0.8
F820-4-D2-IR		HLCVLEELFWGASLFGYCSG	21.5	4.0	2.3	1.8 / 0.6
F820-4-C5-IR		HLCVLEELFWGASLFGYCSG				

Figure 4D (Cont)

Clone Design	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGFsR	IR	IGF/IR	IR/IGF	
A6L-3-C4-IR	HLCVLEELFWGASLFGYCSCG	36.9	1.0	40.5	<0.1	<0.1	42.5
A6L-3-D7-IR	HLCVLEERFWGASLFGQCSCG	38.6	1.0	40.1	<0.1	<0.1	40.7
A6L-3-A1-IR	QLCVLEELHWGASLFGYCSCG	39.6	1.1	44.8	<0.1	<0.1	40.6
A6L-3-C1-IR	PLCVLEBPQFWGASLFGQCSCG	37.3	1.0	40.3	<0.1	<0.1	40.3
A6L-3-D5-IR	YLCVLEERFWGASLFGQCSCG	42.9	1.1	44.4	<0.1	<0.1	40.2
A6L-3-A4-IR	HLCVLEELFWGASLFGQCSCG	26.7	1.1	42.2	<0.1	<0.1	40.2
A6L-3-D3-IR	HLCVLEERFWGASLFGQCSCG	34.6	0.9	36.9	<0.1	<0.1	39.8
A6L-3-B1-IR	HLCVMEELPWGTSLFGQCCTG	33.9	1.0	38.7	<0.1	<0.1	39.3
A6L-3-B5-IR	HLCVLEERFWGASLFGQCSCG	35.3	1.1	42.4	<0.1	<0.1	38.6
A6L-3-B2-IR	HLCVLEERFWGASLFGQCSCG	38.1	1.1	42.7	<0.1	<0.1	37.7
B6H-4-G12-IR	HLCVLEELFWGASLFGQCSCG	31.6	1.1	39.6	<0.1	<0.1	36.7
B6C-4-H10-IR	QLCVLEELFWGASLFGQCSCG	38.5	1.1	41.1	<0.1	<0.1	36.5
B6H-4-G8-IR	HLCVLEEMFWGASLFGQCSCG	31.7	1.1	39.7	<0.1	<0.1	36.2
A6L-3-D6-IR	HLCVLEELFWGASLFGQCSCG	35.5	1.0	37.2	<0.1	<0.1	36.1
B6C-4-F1-IR	QLCVLEELFWGASLFGQCSCG	32.9	1.1	38.7	<0.1	<0.1	35.8
B6C-4-H3-IR	QLCVLEBPQFWGASLFGQCSCG	37.4	1.2	40.5	<0.1	<0.1	34.8
B6H-4-E8-IR	QLCVLEELFWGASLFGYCSCG	30.2	1.0	35.7	<0.1	<0.1	34.3
B6C-4-G1-IR	HLCVLEERFWGASLFGQCSCG	34.9	1.2	40.2	<0.1	<0.1	33.7
B6H-4-E9-IR	HLCVLEERFWGASLFGQCSCG	34.4	1.2	38.8	<0.1	<0.1	33.2
B6C-4-F5-IR	QLCVLEELFWGASLFGYCSCG	34.7	1.2	39.6	<0.1	<0.1	32.8
B6C-4-F11-IR	HLCVLEELFWGASLFGQCSCG	34.0	1.2	37.2	<0.1	<0.1	31.7
B6C-4-E6-IR	HLCVLEELFWGASLFGQCSCG	32.3	1.2	37.4	<0.1	<0.1	30.6
B6C-4-E12-IR	HLCVLEELFWGASLFGQCSCG	30.9	1.1	33.3	<0.1	<0.1	30.2
B6C-4-G10-IR	HLCVLEELFWGASLFGQCSCG	33.0	1.3	40.3	<0.1	<0.1	30.1
B6C-4-F8-IR	QLCVLEBPQFWGASLFGQCSCG	36.4	1.4	39.8	<0.1	<0.1	29.3
20C-3-B5-IR	HLCVLEERFWGASLFGQCSCG	26.6	1.1	32.5	<0.1	<0.1	29.2
B6C-4-G3-IR	HLCVLEEMFWGASLFGQCSCG	34.0	1.4	38.8	<0.1	<0.1	28.3
20C-3-B7-IR	PLCVLEELFWGASLFGQCSCG	29.5	1.2	32.9	<0.1	<0.1	28.3

Figure 4E

Clone	Parental/Design	Sequence	Ratios over Background			Comparisons		
			E-Tag	ICFSR	IR	ICFR/IR	IR/ICFR	IR/IRFR
D815-4-A8-IR		WLDQENAWQCEVYGRGCP	44.8	1.4	24.2	<0.1	17.3	
D815-4-A8-IR		WLDQENAWQCEVYGRGCP	48.0	1.0	48.4	<0.1	48.4	
D815-4-D10-IR		WLDQENAWQCEVYGRGCP	49.2	1.0	48.2	<0.1	48.2	
D815-4-D9-IR		WLDQENAWQCEVYGRGCP	47.5	1.0	48.0	<0.1	48.0	
D815-4-A11-IR		WLDQENAWQCEVYGRGCP	47.9	1.0	48.0	<0.1	48.0	
D815-4-E12-IR		WLDQENAWQCEVYGRGCP	49.0	1.0	47.6	<0.1	47.6	
D815-4-B7-IR		WLDQENAWQCEVYGRGCP	45.4	1.0	47.2	<0.1	47.2	
D815-4-D11-IR		WLDQENAWQCEVYGRGCP	49.5	1.0	47.0	<0.1	47.0	
D815-4-D12-IR		WLDQENAWQCEVYGRGCP	48.1	1.0	46.6	<0.1	46.6	
D815-4-F8-IR		WLDQENAWQCEVYGRGCP	47.8	1.0	46.4	<0.1	46.4	
D815-4-A9-IR		WLDQENAWQCEVYGRGCP	47.7	1.0	45.8	<0.1	45.8	
D815-4-E9-IR		WLDQENAWQCEVYGRGCP	47.8	1.0	45.8	<0.1	45.8	
D815-4-B10-IR		WLDQENAWQCEVYGRGCP	49.0	1.0	45.6	<0.1	45.6	
D815-4-H8-IR		WLDQENAWQCEVYGRGCP	49.0	1.0	45.6	<0.1	45.6	
D815-4-E10-IR		WLDQENAWQCEVYGRGCP	47.0	1.0	45.6	<0.1	45.6	
D815-4-G9-IR		WLDQENAWQCEVYGRGCP	44.5	1.0	44.2	<0.1	44.2	
D815-4-D7-IR		WLDQENAWQCEVYGRGCP	44.2	1.0	44.2	<0.1	44.2	
D815-4-G12-IR		WLDQENAWQCEVYGRGCP	44.3	1.0	43.7	<0.1	43.7	
D815-4-E11-IR		WLDQENAWQCEVYGRGCP	45.5	1.0	43.0	<0.1	43.0	
D815-4-H7-IR		WLDQENAWQCEVYGRGCP	46.2	1.0	43.0	<0.1	43.0	
D815-4-F12-IR		WLDQENAWQCEVYGRGCP	47.2	1.0	42.6	<0.1	42.6	
D815-4-E8-IR		WLDQENAWQCEVYGRGCP	47.9	1.0	42.6	<0.1	42.6	
D815-4-F9-IR		WLDQENAWQCEVYGRGCP	46.4	1.0	41.8	<0.1	41.8	
D815-4-A10-IR		WLDQENAWQCEVYGRGCP	47.3	1.0	41.2	<0.1	41.2	
D815-4-C7-IR		WLDQENAWQCEVYGRGCP	37.7	1.0	40.0	<0.1	40.0	
D815-4-H10-IR		WLDQENAWQCEVYGRGCP	47.0	1.0	39.8	<0.1	39.8	
D815-4-C9-IR		WLDQENAWQCEVYGRGCP	44.2	1.0	39.8	<0.1	39.8	
D815-4-F11-IR		WLDQENAWQCEVYGRGCP	40.4	1.0	39.2	<0.1	39.2	
D815-4-H12-IR		WLDQENAWQCEVYGRGCP	45.4	1.0	38.6	<0.1	38.6	
D815-4-A7-IR		WLDQENAWQCEVYGRGCP	37.3	1.0	37.3	<0.1	37.3	
D815-4-H11-IR		WLDQENAWQCEVYGRGCP	2.4	1.0	37.2	<0.1	37.2	
D815-4-F7-IR		WLDQENAWQCEVYGRGCP	32.4	1.0	34.7	<0.1	34.7	

Figure 6B

Clone	Parental/Design	Sequence	Ratios over Background		Comparisons	
			E-Tag	IR	IGFR/IR	IR/IGFR
		WLDQEWAWVQCEVYVGRGCPSS	--	--	--	--
D815-4-G8-IR		QLDQEWAVRCEVWGRGCPSS	27.8	1.0	33.6	<0.1
D815-4-G7-IR		WLDLEWAQVQCKVYVGRGCPSS	34.7	1.0	32.3	<0.1
D815-4-G11-IR		WLDLEWAQVQCKVYVGRGCPSS	30.7	1.0	28.6	<0.1
D815-4-E7-IR		WLDQEWAWVQCEVWGRGCPSS	33.0	1.0	26.4	<0.1
D815-4-A12-IR		WLDREWAQVQCEVYVGRGCLSS	28.4	1.0	19.0	0.1
D815-4-B11-IR		WLDREWEWVQCEVYVGRGCRP	22.1	1.0	18.8	0.1
D815-4-D8-IR		SILDRWAVVQCVYVGRGCPSS	20.8	1.0	14.6	0.1

Figure 6B (Con't)

Clone	Parental/Design	Sequence	Ratios over Background			Comparisons	
			E-Tag	IGF5R	IR	IGF5R	IR/IGF5R
D820-3-H2-IR		WLDQENAWTQCEVYGRGCP	44.8	1.4	24.2	0.1	17.2
D820-3-H2-IR		RDLEENAWTQCEVYGRGCP	23.9	1.0	40.0	<0.1	40.0
D820-3-C4-IR		WLEQENAWTQCEVYGRGCS	31.0	1.0	39.5	<0.1	39.5
D820-3-C3-IR		WLEQENAWTQCEVYGRGCP	35.2	1.0	39.4	<0.1	39.4
D820-3-G6-IR		WLEQENAWTQCEVYGRGCS	33.8	1.0	38.8	<0.1	38.8
D820-3-D2-IR		WLDQENAWTQCEVYGRGCP	35.6	1.0	37.8	<0.1	37.8
D820-3-D3-IR		WLDQENAWTQCEVYGRGCS	34.8	1.0	37.7	<0.1	37.7
D820-3-B5-IR		WLEQENAWTQCEVYGRGCP	34.1	1.0	37.1	<0.1	37.1
D820-3-E2-IR		C7EQENGLVQCEVYGRGCP	34.4	1.0	37.0	<0.1	37.0
D820-3-B3-IR		WLEQENAWTQCEVYGRGCS	33.6	1.0	36.7	<0.1	36.7
D820-3-B6-IR		WLEQENAWTQCEVYGRGCP	31.2	1.0	36.6	<0.1	36.6
D820-3-D4-IR		WLEQENAWTQCEVYGRGCP	32.0	1.0	36.2	<0.1	36.2
D820-3-C2-IR		WLEQENAWTQCEVYGRGCS	33.7	1.0	35.6	<0.1	35.6
D820-3-F6-IR		WLEQENAWTQCEVYGRGCS	30.8	1.0	35.2	<0.1	35.2
D820-3-D5-IR		WLEQENAWTQCEVYGRGCP	30.5	1.0	34.8	<0.1	34.8
D820-3-F5-IR		WLEQENAWTQCEVYGRGCS	29.8	1.0	34.6	<0.1	34.6
D820-3-H3-IR		WLEQENAWTQCEVYGRGCS	30.2	1.0	33.8	<0.1	33.8
D820-3-G2-IR		WLEQENAWTQCEVYGRGCP	31.3	1.0	33.0	<0.1	33.0
D820-3-H6-IR		WLEQENAWTQCEVYGRGCP	30.3	1.0	32.2	<0.1	32.2
D820-3-F3-IR		WLEQENAWTQCEVYGRGCA?	28.6	1.0	30.7	<0.1	30.7
D820-3-B4-IR		WLEQENAWTQCEVYGRGCS	33.1	1.0	30.5	<0.1	30.5
D820-3-C5-IR		WLEQENAWTQCEVYGRGCP	29.1	1.0	30.3	<0.1	30.3
D820-3-F4-IR		WLEQENAWTQCEVYGRGCS	25.9	1.0	29.5	<0.1	29.5
D820-3-H5-IR		WLEQENAWTQCEVYGRGCS	26.3	1.0	28.6	<0.1	28.6
D820-3-A6-IR		WLEQENAWTQCEVYGRGCP	24.8	1.0	26.0	<0.1	26.0
D820-3-A2-IR		WLEQENAWTQCEVYGRGCP	23.7	1.0	25.6	<0.1	25.6
D820-3-G5-IR		WLEQENAWTQCEVYGRGCS	22.6	1.0	25.0	<0.1	25.0
D820-3-G3-IR		WLEQENAWTQCEVYGRGCS	22.2	1.0	23.9	<0.1	23.9
D820-3-E3-IR		WLEQENAWTQCEVYGRGCS	20.6	1.0	22.7	<0.1	22.7

Figure 6C

Clone Parental/Design	Sequence	Ratios over Background			Comparisons	
		E-Tag 44.8	IGFsR	IR	IGFR/IR	IR/IGFR
D820-3-D5-IGFR	WLDQENAWQCEVYGRGCPES	44.8	1.4	24.2	0.1	17.3
D820-3-E4-IGFR	WYNQALGVQSDVQGRRCOS	29.6	3.8	1.0	3.8	0.3
D820-3-E5-IGFR	LLDHENPWGCEVYGRGSL	27.1	3.2	1.0	3.2	0.3
D820-3-F4-IGFR	WLHQELAWRGEGYPRGRES	25.0	3.1	1.0	3.1	0.3
D820-3-F6-IGFR	WLGHDNANTQCEVYGLGCEP	3.9	2.7	1.0	2.7	0.4
D820-3-G4-IGFR	WIDQEGVRQCEA*GRAFES	26.7	2.6	1.0	2.6	0.4
D820-3-E2-IGFR	WRDEENAWQGVQVQGRGMPA	3.8	2.6	1.0	2.6	0.4
D820-3-G6-IGFR	RLGVENSFORKVYGRDSTS	15.3	2.6	1.0	2.6	0.4
D820-4-E11-IGFR	WLAQGNAGVQCVYVYGRGCRN	20.3	2.4	1.0	2.4	0.4
D820-4-H11-IGFR	WLEEE*AGIQCVYGRGCP	12.6	1.0	3.0	0.3	3.0
D820-4-D11-IGFR	WLDQENWQCEVWGRGCL	8.1	1.0	4.6	0.2	4.6
D820-4-A8-IGFR	RLQENALIQCEVYGRGCP	4.5	1.0	5.3	0.2	5.3
D820-4-F9-IGFR	WLEBENAWQCVYVYGRGCA	3.2	1.0	5.5	0.2	5.5
D820-4-C8-IGFR	WLDLE*EMIQCEVYGRGCAI	9.4	1.0	5.8	0.2	5.8
D820-4-D9-IGFR	WLDQENWQVQCEVYGRGCP	11.6	1.0	5.9	0.2	5.9
D820-4-D7-IGFR	WLEBENAWQCEVYGRGCP	10.1	1.0	8.9	0.1	8.9
D820-4-H9-IGFR	WLDQENAWQCEVWGRGCTY	34.1	3.5	33.4	0.1	9.5
D820-4-E10-IGFR	YLD7ENAWQCEVYGLGCO	18.4	1.0	10.1	0.1	10.1
D820-4-E7-IGFR	WLDVE*AWQCEVWGRGCP	26.7	2.6	27.0	0.1	10.4
D820-4-H8-IGFR	WLEQENWQCEVYGRGCP	31.9	3.0	32.2	0.1	10.7
D820-4-A11-IGFR	WLEBENAWQCEVYGRGCL	16.1	1.0	11.7	0.1	11.7
D820-4-C9-IGFR	WLDQENAWIQCEVYGRGCP	8.0	1.0	12.5	0.1	12.5
D820-4-E9-IGFR	?LEBENAWIQCEVYGRGCO	19.6	1.0	14.9	0.1	14.9
D820-4-B10-IGFR	WLD7ENAWIQCEVYGRGCP	19.3	1.0	17.3	0.1	17.3
D820-4-F10-IGFR	WLD7ENAWQCEVYGRGCP	19.3	1.0	21.5	<0.1	21.5
D820-4-B9-IGFR	GLEQGC*PWGLEVQVGRGCP	25.7	1.0	25.7	<0.1	25.7
D820-4-G8-IGFR	WLEBENAWQCEVYGRGCP	31.7	1.0	26.5	<0.1	26.5
D820-4-G8-IGFR	WLDQENAWIQCEVYGRGCS	25.6	1.0	29.3	<0.1	29.3

Figure 60

Clone	Parental/Design	Sequence	Ratios over Background			Comparisons	
			E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
D820-4-G9-IGFR		WLDQENAWQCEVYGRGCP	44.8	1.4	24.2	0.1	17.3
D820-4-C10-IGFR		WLDQENAWQCEVYGRGCP	36.8	1.0	29.6	<0.1	29.6
D820-4-A9-IGFR		WLDLEWVFQCEVYGRGCP	32.6	1.0	31.3	<0.1	31.3
D820-4-B8-IGFR		WLEQENAWQCEVYGRGCP	20.4	1.0	31.4	<0.1	31.4
D820-4-F8-IGFR		WLDLEWQIKCKVYGRGCP	31.1	1.0	32.7	<0.1	32.7
D820-4-H7-IGFR		WLEQENAWQCEVYGRGCP	28.3	1.0	32.9	<0.1	32.9
D820-4-E8-IGFR		WLEQENAWQCEVYGRGCP	34.1	1.0	32.9	<0.1	32.9
D820-4-G10-IGFR		WLEQENAWQCEVYGRGCP	26.6	1.0	33.2	<0.1	33.2
D820-4-D10-IGFR		WLE?EWQCEVYGRGCP	37.5	1.0	33.2	<0.1	33.2
D820-4-D8-IGFR		WLEQENAWQCEVYGRGCP	36.6	1.0	33.5	<0.1	33.5
D820-4-A10-IGFR		WLEQENAWQCEVYGRGCP	23.7	1.0	34.6	<0.1	34.6
D820-4-B7-IGFR		WLEQENAWQCEVYGRGCP	29.4	1.0	35.5	<0.1	35.5
D820-4-E12-IGFR		WLEQENAWQCEVYGRGCP	35.4	1.0	36.9	<0.1	36.9
D820-4-F12-IGFR		WLEQENAWQCEVYGRGCP	37.0	1.0	37.0	<0.1	37.0
D820-4-G12-IGFR		WLEQENAWQCEVYGRGCP	36.8	1.0	37.1	<0.1	37.1
D820-4-H10-IGFR		WLEQENAWQCEVYGRGCP	36.9	1.0	37.3	<0.1	37.3
D820-4-F7-IGFR		WLEQENAWQCEVYGRGCP	34.4	1.0	37.5	<0.1	37.5
D820-4-G12-IGFR		WLEQENAWQCEVYGRGCP	30.3	1.0	37.8	<0.1	37.8
D820-4-D12-IGFR		WLEQENAWQCEVYGRGCP	37.2	1.0	38.6	<0.1	38.6
D820-4-A12-IGFR		WLEQENAWQCEVYGRGCP	30.4	1.0	39.3	<0.1	39.3
D820-4-C12-IGFR		WLEQENAWQCEVYGRGCP	37.1	1.0	39.6	<0.1	39.6
D820-4-A7-IGFR		WLEQENAWQCEVYGRGCP	35.4	1.0	40.8	<0.1	40.8
D820-4-B12-IGFR		WLEQENAWQCEVYGRGCP	36.2	1.0	41.4	<0.1	41.4

Figure 6D (Con't)

Clone	Parental/Design	Sequence IR/IGFR	Ratios over Background		Comparisons	
			E-Tag	ICFs	IR	IGFR/IR
B6-4-G12-IR B6-3-A11-IR		<u>WLDQENAWTCCEVYGRGCP</u>	44.8	1.4	24.2	<0.1
		<u>WLDQENAWTCCEVYGRGCP</u>	4.4	1.0	6.9	0.1
		<u>WLDQENAWTCCEVYGRGCP</u>	7.3	1.0	6.3	0.2

Figure 6E

	Sequence	HIR affinity mol/l
J228	HPPLEHLKAFLI-NH ₂	$2.4 \cdot 10^{-5}$
J229	APTFYAWFNQQT-NH ₂	$2.4 \cdot 10^{-6}$
S122	HPTSKEIYAKLLK	$9.3 \cdot 10^{-6}$
S123	HPSTNQMLMKLFLK	$1.6 \cdot 10^{-5}$
S124	HPPLSELKFLIKK	$2.3 \cdot 10^{-5}$

Figure 7

J-nr	Sequence	HIR affinity mol/l
J101	ACVWPTYWNCG	$5.0 \cdot 10^{-6}$
J103	Ac-CVWPTYWNCG	$3.0 \cdot 10^{-5}$
J104	Bz-CVWPTYWNCG	$3.2 \cdot 10^{-5}$
J105	Ac-ACVWPTYWNCG	$4.5 \cdot 10^{-5}$
J109	ACVWPTYWACG	$2.0 \cdot 10^{-5}$
J110	ACVWPTYANCG	$2.4 \cdot 10^{-5}$
J111	ACVWPTAWNCG	$3.1 \cdot 10^{-5}$
J112	ACVWPAYWNCG	$3.3 \cdot 10^{-5}$
J113	ACVWATYWNCG	$5.5 \cdot 10^{-5}$
J115	ACAWPTYWNCG	$2.7 \cdot 10^{-6}$
J116	AAWPTYWNAG	$3.4 \cdot 10^{-5}$
J117	ASVWPTYWNSG	$2.9 \cdot 10^{-5}$
J118	ACPYNWWTWCG	$2.9 \cdot 10^{-5}$
J119	ACVWPTYWnCG	$3.2 \cdot 10^{-5}$
J120	ACVWPTYwNCG	$3.4 \cdot 10^{-5}$
J121	ACVWPTYWNCG	$1.8 \cdot 10^{-5}$
J122	ACVWPtYWNCG	$5.1 \cdot 10^{-5}$
J123	ACVWpTYWNCG	$2.5 \cdot 10^{-5}$
J124	ACVwPTYWNCG	$2.0 \cdot 10^{-5}$
J125	ACvWPTYWNCG	$1.8 \cdot 10^{-5}$
J127	acvwptywncg	$4.4 \cdot 10^{-5}$
J128	gcnwtpwvca	$5.3 \cdot 10^{-5}$
J130	AEVWPTYWN(Dpr)G	$1.9 \cdot 10^{-5}$
J131	ACdWPTYWNCG	$5.5 \cdot 10^{-5}$
J132	AC(Leu)WPTYWNCG	$4.5 \cdot 10^{-6}$
J133	AC(dLeu)WPTYWNCG	$2.8 \cdot 10^{-5}$
J134	AC(Ile)WPTYWNCG	$7.4 \cdot 10^{-6}$
J135	AC(dIle)WPTYWNCG	$2.9 \cdot 10^{-5}$
J136	AC(Met)WPTYWNCG	$7.5 \cdot 10^{-6}$

FIGURE 8

J137	AC(dMet)WPTYWNCG	$2.5 \cdot 10^{-5}$
J138	AC(Abu)WPTYWNCG	$7.8 \cdot 10^{-5}$
J139	AC(dAbu)WPTYWNCG	$2.1 \cdot 10^{-5}$
J140	AC(Nva)WPTYWNCG	$3.6 \cdot 10^{-6}$
J141	AC(dNva)WPTYWNCG	$3.0 \cdot 10^{-5}$
J142	AC(tBuG)WPTYWNCG	$3.2 \cdot 10^{-5}$
J143	AC(dtBuG)WPTYWNCG	$3.8 \cdot 10^{-5}$
J144	AC(Phe)WPTYWNCG	$5.1 \cdot 10^{-6}$
J145	AC(dPhe)WPTYWNCG	$5.7 \cdot 10^{-5}$
J146	AC(Cha)WPTYWNCG	$2.2 \cdot 10^{-5}$
J147	AC(dCha)WPTYWNCG	$1.7 \cdot 10^{-5}$
J148	AC(Nal(1))WPTYWNCG	$5.8 \cdot 10^{-6}$
J149	AC(dNal(1))WPTYWNCG	$2.0 \cdot 10^{-5}$
J150	AC(Acy)WPTYWNCG	$2.0 \cdot 10^{-5}$
J151	ACVWPT(Hyp)WNCG	$2.2 \cdot 10^{-4}$
J154	ACVWPT(Nal2)WNCG	$8.2 \cdot 10^{-5}$
J155	ACVWPT(MetO ₂)WNCG	$1.9 \cdot 10^{-4}$
J157	ACVWPT(Cha)WNCG	$1.2 \cdot 10^{-4}$
J160	ACVWPT(Ser)WNCG	$1.8 \cdot 10^{-4}$
J162	ACVWPT(Thi)WNCG	$2.5 \cdot 10^{-4}$
J163	ACVWPT(dSer)WNCG	$5.0 \cdot 10^{-5}$
J166	ACVWPT(dCha)WNCG	$7.5 \cdot 10^{-5}$
J170	ACVWPT(dPhe)WNCG	$1.4 \cdot 10^{-4}$
J171	ACVWPT(Thr)WNCG	$7.7 \cdot 10^{-4}$
J174	ACVWPT(Phe)WNCG	$4.5 \cdot 10^{-5}$
J176	ACVWPT(dThr)WNCG	$2.8 \cdot 10^{-5}$
J180	ACVWPTYW D CG	$5.6 \cdot 10^{-5}$
J182	ACVWPT D WNCG	$2.7 \cdot 10^{-5}$
J183	ACVWP D YWNCG	$3.3 \cdot 10^{-5}$
J184	ACVW D TYWNCG	$6.2 \cdot 10^{-5}$

Figure 8 (Con't)

J185	ACV d PTYWNCG	$3.4 \cdot 10^{-5}$
J186	AC d WPTYWNCG	$3.5 \cdot 10^{-5}$
J187	ACVWTYWNPCG	$4.3 \cdot 10^{-5}$
J188	ACVWTYWPNCG	$3.0 \cdot 10^{-5}$
J189	ACVWTPWNCG	$3.1 \cdot 10^{-5}$
J190	ACVWTPYWNCG	$2.6 \cdot 10^{-5}$
J191	ACVPWTYWNCG	$3.0 \cdot 10^{-5}$
J192	ACPWWTYWNCG	$4.2 \cdot 10^{-5}$
J193	ACWPTYWNVCG	$4.8 \cdot 10^{-5}$
J194	ACPTYWNVWCG	$4.2 \cdot 10^{-5}$
J195	ACTYWNVWPCG	$3.3 \cdot 10^{-5}$
J196	ACYWNVWPTCG	$2.4 \cdot 10^{-5}$
J197	ACWNWPTYCG	$2.9 \cdot 10^{-5}$
J198	ACNVWPTYWCG	$4.2 \cdot 10^{-5}$
J199	ACVWPCG	$4.7 \cdot 10^{-5}$
J200	CVWPTYWNCG	$5.5 \cdot 10^{-5}$
J201	ACWWPTYWNCG	$6.8 \cdot 10^{-6}$
J202	ACEWPTYWNCG	$4.6 \cdot 10^{-6}$
J203	ACRWPTYWNCG	$5.8 \cdot 10^{-6}$
J204	ACQWPTYWNCG	$9.2 \cdot 10^{-6}$
J205	ACGWPTYWNCG	$4.4 \cdot 10^{-6}$
J207	cyclo-Valeroyl-AWPTYWNCG	$5.5 \cdot 10^{-5}$
J208	cyclo-ToluyI- AWPTYWNCG	$7.6 \cdot 10^{-5}$
J209	cyclo-Acetyl- AWPTYWNCG	$7.7 \cdot 10^{-5}$
J210	(WPTYWNCG) ₂	$5.3 \cdot 10^{-5}$
J211	(AWPTYWNCG) ₂	$7.9 \cdot 10^{-6}$
J212	ACA(Bpa)PTYWNC GK(biotin	$1.8 \cdot 10^{-5}$
J213	ACAWPTY(Bpa)NC GK(biotin	$1.8 \cdot 10^{-5}$
J214	GCAWPTYWNCG	$1.4 \cdot 10^{-6}$
J215	NCAWPTYWNCG	$9.0 \cdot 10^{-6}$

Figure 8 (Con't)

J216	VCAWPTYWNCG	$2.8 \cdot 10^{-6}$
J227	SFYEAHQLLGV-NH ₂	$6.4 \cdot 10^{-6}$
J228	HPPLEHLKAFLL-NH ₂	$2.4 \cdot 10^{-5}$
J229	APTFYAWFNQQT-NH ₂	$2.4 \cdot 10^{-6}$
S122	HPTSKEIYAKLLK	$9.3 \cdot 10^{-6}$
S123	HPSTNQMLMKLFK	$1.6 \cdot 10^{-5}$
S124	HPPLSELKFLIKK	$2.3 \cdot 10^{-5}$

Figure 8 (Con't)

Clone	Sequence	Ratios over Background		Comparisons	
		E-Tag	IR	IGFR/IR	IR/IGFR
H5 Parental	LCQSLGVTPGWLGNCA	--	1.2	--	--
H5-3-JBA5-IGFR	LCQSMGVRIGMLAGLCP	31.9	16.3	--	--
H5-3-E1A11-IGFR	VCQSLGITDLGLCAGWA	21.3	8.0	--	--
H5-3-E4B10-IGFR	LCQSLGLTHPGFAWLCA	29.7	7.8	--	--
H5-3-E4C10-IGFR	LCQNFVTDPCFYGNFA	24.3	6.1	--	--
H5-3-JBB6-IGFR	PCQRLGDTHLCWLGNFA	40.2	5.4	--	--
H5-3-E4A9-IGFR	LCQSSGLSFLGCLGNWA	27.7	4.3	--	--
H5-3-E2A12-IGFR	LCQSLGFTDLMLACNFE	27.2	4.2	--	--
H5-3-E4A12-IGFR	VCQGLGVPCGWFAGNWA	27.9	3.9	--	--
H5-3-E1F9-IGFR	PCQSLGLTCSNFEHGNA	18.6	3.5	--	--
H5-3-E4F11-IGFR	LCQQWGIIRIGMLVGRCM	28.4	3.3	--	--
H5-3-E4A11-IGFR	LMQSVGIKYPGGLAGMLA	31.0	3.0	--	--
H5-3-E4G7-IGFR	QMQSLGVTCPGSWAELCA	26.2	2.2	--	--
H5-3-E1B9-IGFR	LCQSLGVTYWESGLAWLCA	20.0	2.1	--	--

Figure 9A

Clone	Sequence	Ratios over Background		Comparisons	
		E-Tag	IR	IGFR/IR	IR/IGFR
JBA5 Parental	LCQSMGVRIQWLAGLCP	31.5	20.6	1.0	20.6 <0.1
JBA5-4-2C12-IGFR	LCQSMGVRIQWLAGLCP	46.8	41.5	1.0	41.5 <0.1
JBA5-2-1F9-IGFR	LCESMGVRIQWLAGLCP	48.1	39.5	1.0	39.5 <0.1
JBA5-2-1E10-IGFR	LCQSMGVRIQWLAGLCP	42.5	39.5	1.1	35.9 <0.1
JBA5-4-2A11-IGFR	LCQSMGVRIQWLAGLCP	44.1	40.2	1.2	33.5 <0.1
JBA5-3-2A3-IGFR	LCQSMGVRIQWLAGLCP	34.7	33.3	1.0	33.3 <0.1
JBA5-4-2A9-IGFR	LCQSMGVRIQWLAGLCP	34.6	33.1	1.0	33.1 <0.1
JBA5-1-1B6-IGFR	MCQSMGVRIQWLAGLCP	39.6	31.4	1.0	31.4 <0.1
JBA5-4-2B9-IGFR	LCQSMGVRIQWLAGLCP	39.6	22.3	1.0	22.3 <0.1
JBA5-1-1H7-IGFR	LCQSMGVRIQWLAGLCP	24.9	22.6	1.2	18.8 0.1
JBA5-3-2C3-IGFR	LCQSMGVRIQWLAGLCP	35.5	15.3	1.1	13.9 0.1
JBA5-1-1G7-IGFR	LCQSMGVRIQWLAGLCP	26.2	14.8	1.5	9.9 0.1
JBA5-2-1E9-IGFR	LCQSMGVRIQWLAGLCP	39.4	4.5	1.0	4.5 0.2
JBA5-2-1D12-IGFR	L*KSMDVRSGLWAGLCP	42.2	2.2	1.0	2.2 0.5

Figure 9B

Clone	Design	Sequence	Ratios over Background		Comparisons	
			E-Tag	IR	IGFR/IR	IR/IGFR
20F-4-B7-IGFR		XXXXXXXXXXXXXXXXXX	--	--	--	--
20F-4-E4-IGFR		TPIPAGGINIASGGYTWS	10.9	3.7	0.5	7.3
20F-4-E12-IGFR		HRGTVTGVMWPGYHWLS	8.9	4.7	0.7	6.3
20F-4-F4-IGFR		SDVWAQQRNDWPGYHWLS	9.7	4.7	0.8	6.0
20F-4-F7-IGFR		HRGTVTGVMWPGYHWLS	13.9	10.1	1.8	5.6
20F-4-E7-IGFR		SDVWAQQRNDWPGYHWLS	13.7	3.9	0.8	5.1
20F-4-F11-IGFR		RPHRINPQDDAVPGYWL	7.2	2.5	0.5	4.7
20F-4-D10-IGFR		HRGTVTGVMWPGYHWLS	17.6	16.2	3.5	4.6
20F-4-B12-IGFR		FGRGDDGGGYSYEWL	9.8	2.4	0.6	4.1
20F-4-A9-IGFR		DGLVVKSGRMPGYWNL	17.3	14.4	3.6	4.0
20F-4-G2-IGFR		DGSIIVSSVSGMPGYWLM	10.1	9.9	2.4	4.0
20F-4-D11-IGFR		WQOANLSNGGRMGYDWLM	6.6	2.7	0.7	4.0
20F-4-G4-IGFR		FGRGDDGGGYSYEWL	5.1	1.3	0.5	2.7
20F-4-G12-IGFR		VNYEMDRVPMWPGYHWLS	5.0	1.0	0.5	2.3
20F-4-G1-IGFR		MGGGLWGVHWPYLSQ	3.9	0.9	0.5	1.8
20F-4-G12-IGFR		SDVWAQQRNDWPGYHWLS	3.2	0.9	0.6	1.5

Figure 10A (Con't)

Clone	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGF3R	IGFR/IR	IR/IGFR
Design	XXXXXXXXXXXXXXXXXX	--	--	--	--
R20-4-F10-IGFR	CLGAGSFRAGILCLGLPVS	35.5	6.0	--	--
R20-4-F7-IGFR	GPWATACGGQICEELGLKP	29.1	4.7	--	--
R20-4-H9-IGFR	DLFCAYMAQALGLQDLSCG	25.7	3.0	--	--
R20-3-A4-IGFR	RHLLLPQIWTAS*GGWGMG	15.6	2.7	--	--

Figure 10E

Clone	Sequence	Ratios over Background			Comparisons		
Design		E-Tag	ICFSR	IR	ICFSR/IR	IR/ICFSR	
20C-3-H3-IGFR	XXXXXXXXXXXXXXXXXXXXX	28.5	26.6	1.0	26.6	<0.1	
20C-3-F4-IGFR	DHRLCGTDEYLMQDLFVRGLCLRIW	34.4	27.5	1.2	23.1	<0.1	
20C-4-C10-IGFR	GLLFCKQLFTLAGLQPEAGCVSSR	35.5	24.4	1.3	19.2	0.1	
20C-3-G5-IGFR	INTACLDLLRQVWSSCRRAPIG	29.3	21.1	1.1	18.7	0.1	
20C-3-A2-IGFR	DWRLCLVILLSGLTELANTGCVQG	33.9	18.3	1.1	16.9	0.1	
20C-3-B4-IGFR	WFSFCLGGLLQAGWSVWGRDVCCI	39.8	29.1	1.9	15.2	0.1	
20C-3-C6-IGFR	GYSWLRDVLMEKQALKEGSGVRQ	34.8	20.9	1.4	14.9	0.1	
20C-3-E2-IGFR	FLTRLLRLGLS*ERGEAGGPAQA	33.7	14.3	1.2	12.4	0.1	
20C-3-A3-IGFR	FSGFCWGLERLSQVSLGYCGAGQGG	30.2	9.8	0.9	11.2	0.1	
20C-3-B1-IGFR	ISFRQCLFVLAGMHPCPVDVGGEGF	35.5	31.9	3.9	8.2	0.1	
20C-3-F5-IGFR	LQGFCELLATVTGVTGLGCLDYQPI	33.3	19.3	2.8	6.9	0.1	
20C-4-A7-IGFR	GSSICNLLARAQIVELALCEMGVQE	30.5	27.7	5.3	5.2	0.2	
20C-4-F8-IGFR	LSFACLLSGLSGVLPDCLLGED	24.7	13.3	2.8	4.7	0.2	
20C-4-G11-IGFR	GEHPQQLLSLSCGDDGCPVNCGGGS	34.0	5.1	1.6	3.1	0.3	
20C-3-E1-IGFR	GWFECLLASLVLVQFQGRSRASVC	37.3	32.8	13.7	2.4	0.4	
20C-3-B6-IGFR	YRQECACSVAGVGLGLCLARSG						

Figure 10F

Clone	Sequence	Ratios over Background			Comparisons	
Design		E-Tag	IGFSR	IR	IGFR/IR	IR/IGFR
40F-4-D1-IGFR	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX	--	--	--	--	--
40F-4-B1-IGFR	LSCLAYSRHGHWRPSTDLGLGRSVGGGSVSTRWRGYDMFE	4.9	4.6	0.3	13.1	0.1
40F-4-D10-IGFR	GLDHSDAVGVHLGFAMPA.ARGWEAGGLEDTWAGYDWL	4.1	3.0	0.2	13.1	0.1
40F-3-A3-IGFR	W.GYAWLS	4.9	4.5	0.4	11.7	0.1
40F-4-C4-IGFR	LSCLAYSRHGHWRPSTDLGLGRSVGGGSVSTRWRGYDMFE	2.6	2.0	0.3	7.9	0.1
	EAMAVGLQCPARFVRAAAHGGDGSWGQDHV.AMGGYWWLGL	3.8	2.0	0.5	4.1	0.2

Figure 10F (Con't)

Clone	Parental/Design	Sequence	Ratios over Background			Comparisons	
			E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
		<u>HLCVLEELFWGASLFGYCSG</u>	39.1	1.8	27.7	0.1	15.4
	F815-4-G11-IGFR	HRFVIVLERLSGASLFGSSA	34.6	7.9	1.0	7.9	0.1
	F815-3-D1-IGFR	HRFVREGLIWGAYQFCYCSG	14.9	1.0	2.0	0.5	2.0
	F815-4-C12-IGFR	FQSLLEELVWGAPLFRYGTG	35.2	1.0	2.0	0.5	2.0
	F815-4-A11-IGFR	HLSVLEELSWGASLFGQWAG	5.4	1.0	2.1	0.5	2.1

Figure 10G

Clone	Parental/Design	Sequence	Ratios over Background			Comparisons	
			E-Tag	IGFsR	IR	IGF/IR	IR/IGF
NNKH-2-C5-IGFR		HL*VLLEELSMGASLFCQWAG	5.4	1.0	2.1	0.5	2.1
NNKH-2-D9-IGFR		HL*VLLEELSMGASLFCQWAV	7.3	0.9	0.7	1.3	0.8
NNKH-2-D12-IGFR		HL*VLLEEL*LGASMFGLWAG	4.1	0.5	0.4	1.3	0.8
NNKH-2-H10-IGFR		HL*VLLEELSM*ASLFCQWAG	5.0	1.3	1.1	1.2	0.8
NNKH-2-G9-IGFR		HL*SALEELSMGASLFCQWAG	4.8	2.1	1.9	1.1	0.9
NNKH-2-C6-IGFR		HL*SALEEL*CALLFCQWAG	1.9	1.4	1.3	1.1	0.9
NNKH-2-C7-IGFR		RL*SVLEELSMGASLFCQWAG	18.2	1.0	0.9	1.1	0.9
NNKH-2-F11-IGFR		HL*VLVQPSMGASLFCQWAG	21.8	1.3	1.3	1.0	1.0
NNKH-2-H3-IGFR		HQ*SVLEELSR*ASLFCQWAG	6.7	1.3	1.4	0.9	1.1
NNKH-2-B8-IGFR		DMSVLEELSMGA*LFQWAG	4.7	0.7	0.8	0.9	1.1
NNKH-2-B12-IGFR		HL*SVREGQLMFA*SMFCQWAG	17.5	3.7	5.2	0.7	1.4
NNKH-2-F9-IGFR		QL*SVLVEL*MGASLFCQWAA	1.2	1.0	2.9	0.3	2.9
		HL*SVGEELSM*VALLGQWAR	3.7	0.6	2.1	0.3	3.5

Figure 101

D Name	Clonal Name	Formula #	K _d (nM) HIR	PO ₂	Fat Cell Assay	Activity	K _d (nM) HIGFR	Ratio IGF/IR	Sequence
D101	20D3	1	0.51 0.27				13 41	25	KUGGQQHQDGNFYDWFVEALAKK (e-biotin)
D102	20D1	1	1.2 0.97				7.4 16	6.2 16	KVLQAHGCDSSVSDCFYEWFAKK (e-biotin)
D103	1B8	1	0.74				15	20	KWSALLSYMDTGTFVAVFDDAVKK (e-biotin)
D104	E7	1	2.0				>20	>1	KGISWALVHHVDRI FYEWFDLKK (e-biotin)
D105	1B8	1	2.8				12	4.3	KRDKPTDQEQNUSFYEFRLKK (e-biotin)
D106	20F1	1	0.97				6.2	6.4	KFYWCRSQDLFYEFWFFQAARK (e-biotin)
D107	40G11	1	1.1	YES		Antagonist	9.7	8.8	KLESHYVYVQAALDRI FYFSWFKK (e-biotin)
D108	3G11	1	2.3			Antagonist	19	8.3	K FYGWFSRQLSLTRDDDWGLPKK (e-biotin)
D109	20I11	1	3.6			Antagonist	12	3.3	KSAPGLVSNKQXGLFYFSWFREKK (e-biotin)
D110	G3	1	0.84			Antagonist	1.4	1.7	KRGCGITFYEWFSALRKLIGAGKK (e-biotin)
D111	D2	1	0.62				3.2	5.2	KDPERMOSDVG FYEWFFRAAVGKK (e-biotin)
D112	IGFR C1 A654AC1	1	0.49 0.19			Neutral	0.05* 0.02*	0.1 0.1	DYKDCWARPCTGDAAAFYDWFVQVASKK (e-biotin)
D113	IGFR I12 A654-172	1	0.75		~20 nM	Agonist	5.4	7.2	DYKDYVTFITSAVHIENFYDWFVQVSKK (e-biotin)
D114	IGFR A6	1	8.1			Neutral	>20	>2.5	SARNFYDWFVKK (e-biotin)
D115	IGFR D5	1	8.1				>20	>2.5	ADKNFYDWFMAAKK (e-biotin)
D116	IGFR J1B5	9	4.4 cycli		>20 nM	Agonist	8.1	1.8	DYKDLQSQSGVGRIGWLAGLCPKK (e-biotin)
D117	IGFR I12C	1	0.70	YES	~20 nM	Agonist	6.1	8.6	FIENFYDWFVQVSKK (e-biotin)
D118	20E2	2	0.25	YES	~20 nM	Agonist	5.1	8.5	DYKDFYDAIDQLVRGSACAGTRDKK (e-biotin)
D119	20C11	2	0.25	YES	~20 nM	Agonist	1.3 2.5	5.2 0.8	KDRAFYNGLRDLVGA VYGAWDKK (e-biotin)
D120	E8	10	0.37			Antagonist	2.2	5.9	KYRQFGQGTVPFGYEWLRMAAKK (e-biotin)
D121	F2	10	1.1			Antagonist	7.4	6.7	KSMFVAGSDRWFGYGLADWLKK (e-biotin)
D122	20A4 (A7)	6	1.2 1.0			Antagonist	>20 >20	>17 >20	KEIEAEWGRVRLVYGRGVGKK (e-biotin)
D123	D8	6	0.55 1.3			Antagonist	16 29	29 >15	KWLDQEWAWVQCEVYVGRGCPKK (e-biotin)
D124	F8	4	0.64* 0.09*				8.2 >20	200 >200	KHLCVLEELFWGASLFGYCSGKK (e-biotin)
D125	IGFR E4	1	2.6				>20	>8	DYKDERSAAGFRGNFYDWFVAQVNRK (e-biotin)
D126	IGFR D2C	1	1.4				18	13	LGENFYDWFVQVSKK

Figure 11A

Clonal Name	D or S name	Minif	Sequence	IR-Kd	IR-IC ₅₀ Biscore	IR-IC ₅₀ PF-SIT5	PO ₄	Fat Cell Assay
20-42	D118	Ib6	DKKDFYVADQLVRGSARAGTRDK K-biotin	250 nM		2.8 nM	+	++
C1	D112	C6	DYKDFWARPCGDAAFYDFWVQOAS KK-biotin	490 nM			-	0
D8	D121	A6	KWLDQEWAWQCVGVGRGFSKK	550 nM			0	-
F8	D120	GROUP 6	KHGQGGVWVGQYEWI RNA	370 nM			-	-
F8	D124	C-C1 (A6)	KHLVYLHLFWGASLFGYCSGKK	40 nM			-	0
I2C	D117	A6	HIENFYDFWVROQNSKK	700 nM			++	++
KCF9		C-C-C	RIYYEWFWGQIEAQRGGIS			5 nM		
KC-02		Ib6	GLEQCCHWGLEVQRCRPS		<1 µM			
KCG7		Ib6	FYCGLEELSWGAAFGYCSG		2-4 µM		+++	
NG-033		Ib6	GNQDMFYQLSLLVGRDMH		>5 µM	4.2 nM		
NG-08		Ib6	GLSQSCPESEYDFWAGVSDPWWCW					
NG-09		Ib6	VIEGRGLFYDLRLQRLARRNG				-	
RP-1		A6	RANSEYDALNSVLGLGPKK-Biotin		1 µM		+	
RP-2		A6	GRSPVHIEGFYEWFDQLGL		1 µM		+	
RP-3		A6	RSLSASHTVEFYSWFEEQLKS		>10 µM		++	
RP-4		Ib6	GRFYGWFDQADQLMPWGF		5 µM		+	
RP-5		Ib6	PPWGAIFYDAIEQLVFDNL		6 µM		-	
RP-6		C-C	AGVNAGFYRYFTSLLDWWDQGGK-Biotin				++++	
RP-7		A6	TFYSLASLLTGTPQNPGRWERCCK-Biotin					
RP-8		Ib6	AAVHQFYDFWFAIDQYKK		>5 µM		+	
RP-8#	S287	Ib6	QSFYDYHIELLGGEWKK					
RP-9		A6	QSFYDYHIELLGGEWEE			2.9 nM	++	
RP-10		Ib6	GSLDSFYDFWFRQLQKK		>10 µM		+	
RP-11		A6	GSFYDALQRLVGGEQKK		>10 µM		+	
RP-12		Ib6	QAPSNFYDFWVREWDKK					
RP-13		A6	DIHYQGLWEWLRSGKK		>10 µM			
RP-14		A6	ASGPFNFYDFWGRQLSLKK		>10 µM			
RP-15		A6	SACQDFCTENFYDFWFAQKK					
RP-16		Ib6	SOAGSARYAWFDQVLRKYKK					
RP-17		Ib6	VDAQDDYFUISEYTL		>10 µM			
RP-18		Ib6	QSDATYSGLWALGLSDQKK					
RP-19		A6	LQCSGFEYCHERLGVKK					
RP-20		Ib6	LKDFYDFWQRLHLSGKK					
RP-24		GROUP 6	GSASFYDAIDRLRMRIKK					
S167		A6	WPGYLFEEALQDWRGSTED					
S173		Ib6	AFYDFWAKK		No Binding		-	-
S174		Rb6	LDALDRLMYFEERPSL					0
S175		Rb6	PLAFIWAYFEISEGRSSAI		1.2 µM			0
S176		A6	GRDYWLRQANFYDFWVAELG		2-4 µM	0.9 nM	++	++
S176		A6	NGVERAGIGNIFYDFWVAQLH	470 nM				+++

Figure 11B

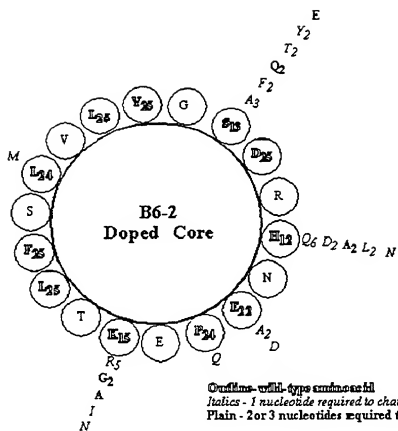


FIGURE 12

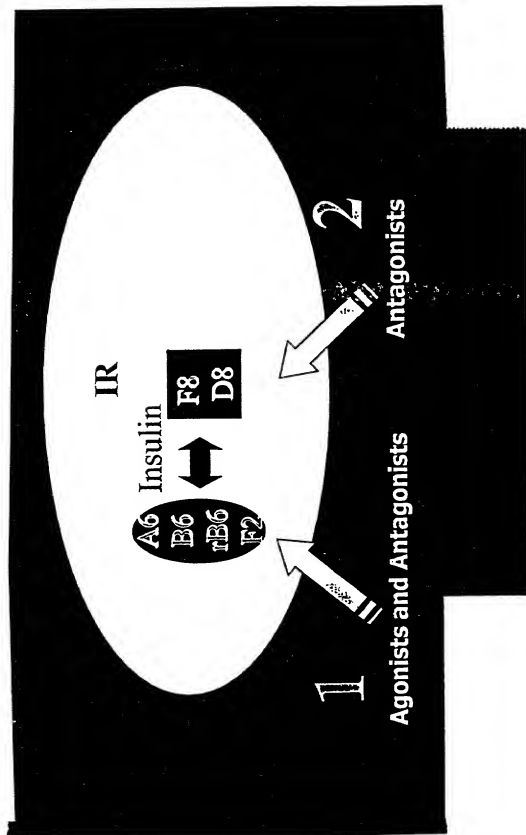


FIGURE 13

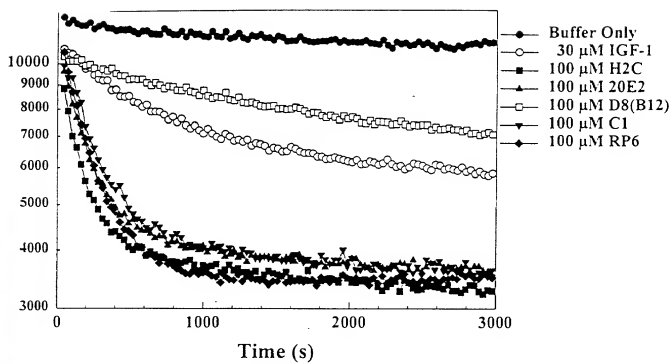


FIGURE 14

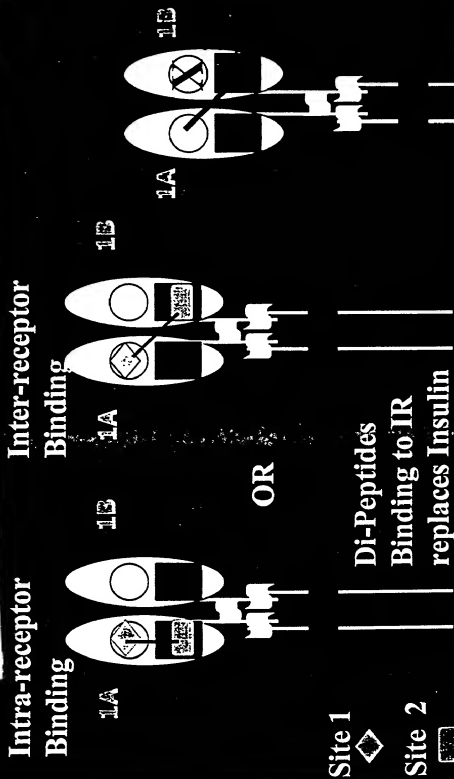


FIGURE 15

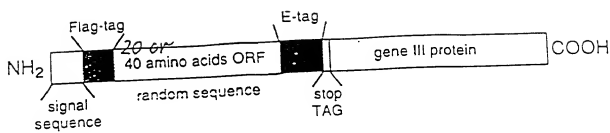


FIGURE 16

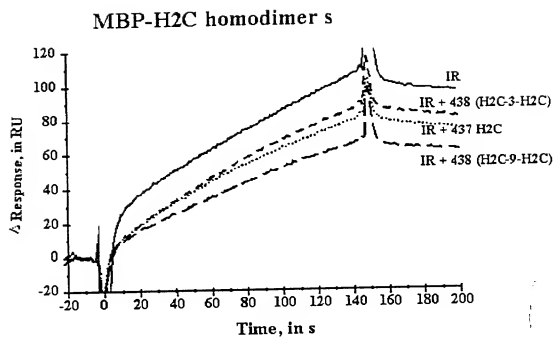


FIGURE 17

Class I clones		# Clones		Competition
		Rnd 3	Rnd 4	
B6 3x	DDKDAETPAQGVGNRLSWSGEGHNWTVDPFFHKLSSELLRESGA	1	2	+
E5 2x	DDKRLHLTNAELGVQSEVLSRLFPDGDIFYRALSHLWRGMGPP	2		+
B5 2x	DDKDRGGMQRQWLDVGARHLERRSVQDNTDDFYGGRLIIVDGF	2		nd
9	DDKDGPPDSFDTVEKGMAILNVRFDPSLDFDNQDTEFYLLDLSL		1	nd
G6	DDYKDGCTYFRGQVAQSNESLLRVNFIQLLEALAASPPT		1	nd
12	DDKDAPLDARLSAPRFOWSPRTWRQSLSYSGEWSFGSDYDCISSI		1	nd
A5	DDYDMGSSQFQDTPRSSQAYSHSLSDSGWGTANWIFRALLEGL		1	nd
C6	DDYKDSGAHEGNOGRSTHILAAINIDHLPDGDAGIWLGSWLS		1	+
Consensus (regular+frameshifters)		d.FY.l1sal		
human IGF mature	s			
	VCGDRGFYFNKPTGYGSSSR	23	30	
human IGF propeptide	MSSSHLFYALCLLFTTSSA	-16	-9	
Class I frameshifting clones (all in +1 frame)				
F6 2x	TTKTRG.IFGMLJGLVLRQILLWFPFKQCVQMDFISLLASL	1	1	+
7 3x	TTKTRIGCCS.LVWGMRGCRLAGDEYAFALWALAG	3		nd
8	TTKTRLRLLLLGGDEPFYGLLRMLIGRS	1		nd
G5	TTKTGFAFW..LAFSVQGVGVAFYSALAALLCAHSASLVCGA	1		nd
Class II clones				
D5	DDYKDPYGGGHIHLYPGTWGVPGFPQKVVLGDADRKNFYDNFM	1		nd
A6	DDYKDYRGMVLVGRISDCAKVASEPPARIGQKVFVNFYDNFV	1		nd
R35	DDYKSGCCRLGLRWNFVIVGWSGALVCQSAASAAGFYDNFV	1		
human IGF mature (1-70)				
GPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPO TGIIVDECCFRSCDLRRLMEYCAPLKPAKSA				

nd = no data

FIGURE 18

IGF-5

GACTACAG ACCGCTGCT GATTTTCGG TACTGTGCG GCGTCTTCA GGTTCAGT TCTCTGCG CCGTCTTCA AGATGTGCT TCAGATGAA GATATTTT ATCTGTGT GGTATGTTG GCGGCCCA 139
D Y K D S M L N F R Y V A G R A Q V S D S V A V S . G L C S D E R Y F F V V G Q T G G R
T T K T R G I F G M L L G V L R F Q I L L W P F P K D C V Q H K D I F Y S L L A L A A
L Q R L V V I F S V C C M A C L G F R F F C G R F L R I V F R . K L F F I R C W L V N R P

IGF-6

GACTACAG ACCGCTGCT GGTCTGCTT GCGTCTGCG GTTTTATGG GTTCTCGT AIGCTGATG GTCGTGGATC TCGGCCCA 180
D Y K D S M L N F R Y V A G R A Q V S D S V A V S . G L C S D E R Y F F V V G Q T G G R
T T K T R G I F G M L L G V L R F Q I L L W P F P K D C V Q H K D I F Y S L L A L A A
L Q R L V V I F S V C C M A C L G F R F F C G R F L R I V F R . K L F F I R C W L V N R P

IGF-05

GACTACAG ACCGCTGCT TTGCTGTGT GTATGATTA GCTTTTTCG TTATAGGGGT GCGTGGCT ITTATCTAG CCGTCTGCG CTCTGTGCT GCGTCTGCG GCGGCCCA 139
D Y K D M L V C L G V M I S F C C G G R F L C C V V C P Q S F F G V M C G G R
T T K T R G I F G M L L G V L R F Q I L L W P F P K D C V Q H K D I F Y S L L A L A A
L Q R L V V I F S V C C M A C L G F R F F C G R F L R I V F R . K L F F I R C W L V N R P

IGF-7

GACTACAG ACCGCTGCT GGTGTGAG CTATGATTT TGGGATGGA GCGATGTAG ATTGCTGCT GGGTTTAT GCTTTTAT GCGTCTGCT GCGGCCCA 112
D Y K D P D M V L Q L I S L G L E G M Q I G . M V L C V F D G A G W G G R
T T K T R I G C C S . L V M G M R G C R L A D G F Y A F L M L A G G A A
L Q R P G L G V A D Q F G V G G D V D M L H G F M R I . M R N L G R P

FIGURE 19

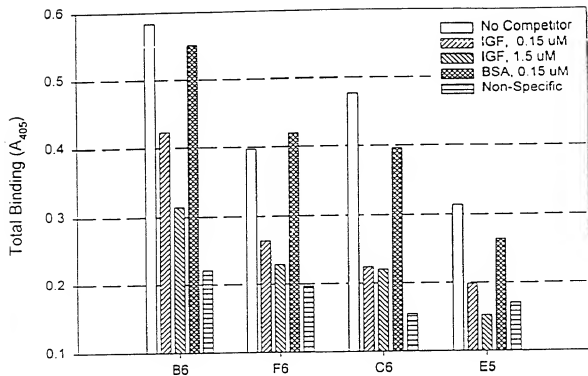


FIGURE 20A

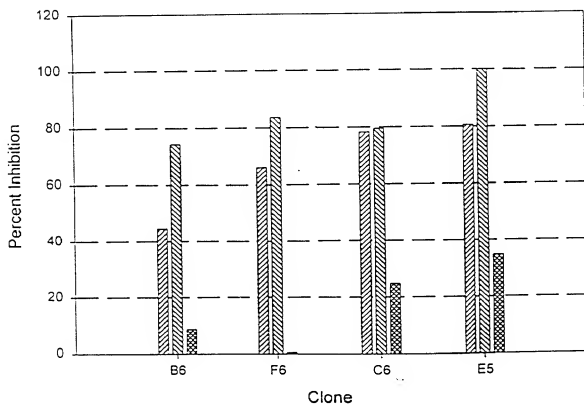


FIGURE 20B

Clone B6
Peptide 5.1 (18 aa)
AETPAQVGNRLWSVPEGEHWNNTVDPFYHKLSELLRESGA
NTVDPFYHKLSELLREKK (biotin)

Clone F6
Peptide 5.2 (17 aa)
MLLGVLRFQILLWFPFKDCVQMKDIFYSLLASL
QMKDIFYSLLASLAACK (biotin)

Clone D5
Peptide 5.3 (14 aa)
PLYGGGIHLYYPGTMGYVPGFPRQVKVLGDADKNFYDWF
ADKNFYDWFMAACK (biotin)

Clone A6
Peptide 5.4 (12 aa)
YRGMVLGRISDGAGKVAPEPPARIGQKVFVNFYDWFV
SAKNFYDWFVKK (biotin)

FIGURE 21

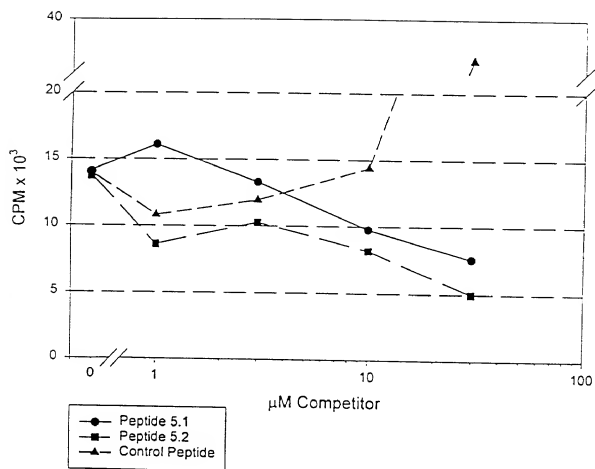


FIGURE 22

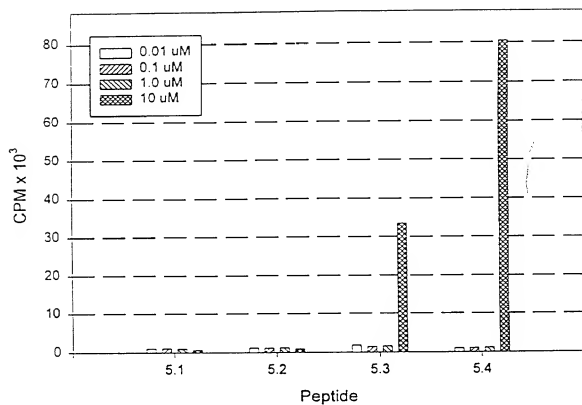


FIGURE 23

FIGURE 24A

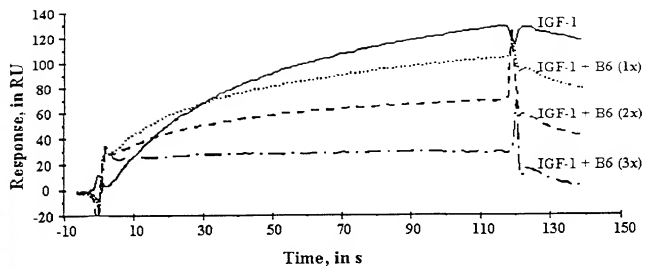


FIGURE 24B

GACTACAAAGACGACGATGACAAGTACCGTGGTATGCTGGTTCGGGTCGTATCTCTGACG
D Y K D D D D K Y R G M L V L G R I S D

GTGCTGGTAAAGTTCCTGAACCGCCGGCTCGTATCGGTTCAGAAAGTTTCGCTGTAA

G A G K V A S E P P A R I G Q K V F A V N

CTTCTACGACTGGTTCGTTGCGGCCGCA 96 nt
F Y D W F V A A A

FIGURE 25A

CTACAAAGACGACGATGACAAGTACCGTGGTATGCTGGTTCGGGTCGTATCTCTGACGGTGTCT
GGTAAAGTTGCTTCTGAACCGCCGGCTCGTATCGGTTCAGAAAGTTTCGCTGTAACTTCTACG
ACTGGTTCGTTGCGGCCGCACTGTGA 154 nt

FIGURE 25B

TTNNKNNKNNKNNK 21 aa
V X X X X

FIGURE 26B

CTACAAAGACNNKNNKNNKNNKNNKNNKNNKNNKNNKNNKAACTTCTACGACTGGTTTCGTtNNK
NNKNNKNNKGCGGCCGCAGTGtGA

Clone:

Binding Ratios:
Target E-Tag % Max

A6S-1-C5	DYKD RIHNQTERCGNFYDWFVHqLV	AAA	7	27	26
A6S-1-G3	DYKD VATVHVGGGMNFYDWFVqVg	AAA	5	19	26
A6S-1-A2	DYKD KDPVTVSQGRNFYDWFVqIQ	AAA	5	20	25
A6S-1-D5	DYKD RVGSGMEDLGNFYDWFVRQAq	AAA	5	25	20
A6S-1-H4	DYKD HKSWTTMSPLNFYDWFVAqVE	AAA	3	18	17
A6S-2-F2	DYKD LAMSVASRPANFYDWFVqIV	AAA	30	35	86
A6S-2-D2	DYKD RAERGSMRDSNFYDWFVqQLP	AAA	30	36	83
A6S-2-E3	DYKD VqEGLSGMEGNFYDWFVDQLF	AAA	28	36	78
A6S-2-H2	DYKD RGqRESDSGTNFYDWFVGAIr	AAA	28	40	70
A6S-2-A3	DYKD SRAPYGSTAGNFYDWFVqAVS	AAA	25	37	68
A6S-2-H1	DYKD RVGIqVDPHTNFYDWFVIQLT	AAA	27	42	64
A6S-2-F1	DYKD VGqVGRYVRSNFYDWFVQqAM	AAA	8	30	27
A6S-2-G1	DYKD RPqLVESGSKNFYDWFVqVVR	AAA	8	30	27
A6S-2-B2	DYKD EMyGDTSERVNFYDWFVSALq	AAA	5	30	17
A6S-2-A1	DYKD LSSRGRVTMRNFYDWFVAqVV	AAA	3	31	10
A6S-3-E1f	DYKD RVREKLPRPENFYDWFVNqIH	AAA	22	23	96
A6S-3-G2	DYKD TWMWEERKqDNFYDWFVqQLK	AAA	20	21	95
A6S-3-E5	DYKD RYRGERHDGRNFYDWFVEqVN	AAA	19	21	90
A6S-3-H2	DYKD qGAEGRLSEGNFYDWFVQAVS	AAA	19	21	90
A6S-3-H9	DYKD YSIEVqDWNENFYDWFVSQLG	AAA	20	23	87
A6S-3-G3	DYKD PRLHMGSDMGDFYDWFVqIA	AAA	18	21	86
A6S-3-F8	DYKD GRGqGLKRPDNFYDWFVAAAK	AAA	20	25	80
A6S-3-G1f	DYKD GAVGLAEAGPNFYDWFVVSqVq	AAA	19	24	79
A6S-3-H1	DYQD PASNKNLSAENFYDWFVqQTR	AAA	23	30	77
A6S-3-E6	DYKD DARDHGVMVMSNFYDWFVqVS	AAA	5	20	25
A6S-3-D9	DYKD SLQGADFqQGNFYDWFVSELA	AAA	4	17	24
A6S-3-E3	DYKD RPSLPEVRPFNFYDWFVqSVR	AAA	4	19	21
A6S-3-H8	DYKD NPTSVqQYGVNFYDWFVNVLS	AAA	4	20	20
A6S-3-G4	DYKD CADPGACSSLNFYDWFVqMRG	AAA	4	21	19
A6S-3-B1f	DYKD YDqDPPYWGLENFYDWFVREVA	AAA	3	16	19
A6S-3-C1	DYKD RPIVIGGGGTRNFYDWFVAqMI	AAA	3	17	18
A6S-4-G5	DYKD QEVTTRRDDKNFYDWFVVSqIF	AAA	26	18	144
A6S-4-D2	DYKD PPRSSRLGENFYDWFVmqVR	AAA	26	19	143
A6S-4-F6	DYKD LKGSSQPLSVNFYDWFVQQIK	AAA	24	17	142
A6S-4-H4	DYKD PRMVEKPSSEDNFYDWFVTqLS	AAA	28	20	141
A6S-4-C1	DYKD CWARPCGDAAANFYDWFVqQAS	AAA	22	16	141
A6S-4-G3	DYKD GAQAIREIHNFYDWFVAQVT	AAA	29	21	139
A6S-4-H3	DYKD GRGDQRHETTIFYDWFVRELq	AAA	28	20	137

FIGURE 28

A6S-4-H6	DYKD	GSIAQLIMRANFYDWFVEqTN	AAA	24	18	130
A6S-4-G8	DYKD	RLMGGIAEFqNFYDWFVREVA	AAA	25	20	126
A6S-4-H5	DYKD	HHSAGNEHGYNFYDWFVLqVA	AAA	24	19	123
A6S-4-E4	DYKD	ERSAAGFREGNFYDWFVAqVN	AAA	32	27	120
A6S-4-F5	DYKD	GSQHSGREPHNFYDWFVAqVG	AAA	28	24	120
A6S-4-D4	DYKD	IARMRETfQPNFYDWFVDQLA	AAA	21	18	118
A6S-4-C6	DYKD	RLDRSSTSGVNFYDWFVAqVG	AAA	28	25	116
A6S-4-D3	DYKD	GLRSEQGNRLNFYDWFVAQIA	AAA	23	20	116
A6S-4-F2	DYKD	SVIQTRQDETnfYDWFV?AMS	AAA	26	23	115
A6S-4-A5	DYKD	VEVQRHIRKDNFYDWFVKQID	AAA	22	19	115
A6S-4-C3	DYKD	VTMLDKGAQDNFYDWFVREVA	AAA	24	21	114
A6S-4-F3	DYKD	HNSSSPMRTGNFYDWFVQELR	AAA	30	26	113
A6S-4-B4	DYKD	ERSPRPALASNFYDWFVQQVV	AAA	21	19	113
A6S-4-B6	DYKD	SDARQAGLQENFYDWFVSQVR	AAA	26	23	113
A6S-4-B1	DYKD	RHERGKEGPGNFYDWFVSQVV	AAA	21	19	112
A6S-4-G4	DYKD	SALSGPVqPINFYDWFVTGM	AAA	30	26	112
A6S-4-A6	DYKD	HVEHMAVGDGNFYDWFVVqLR	AAA	23	21	111
A6S-4-F4	DYKD	VGHSGVPPYPNFYDWFVMQVS	AAA	24	22	110
A6S-4-D6	DYKD	LGAAEtWDGINFYDWFVKQVS	AAA	24	22	110
A6S-4-E6	DYKD	RSSGGLLSqGNFYDWFVSQLE	AAA	26	24	109
A6S-4-A3	DYKD	LAINDLVTHKNFYDWFVDQLR	AAA	20	18	109
A6S-4-E3	DYKD	RGMTGMVGRGNFYDWFVGQLR	AAA	23	21	109
A6S-4-A2	DYKD	IGGQQGHQDGNFYDWFVEALA	AAA	22	20	107
A6S-4-B2	DYKD	QSVDLSRPDSNFYDWFVEVLS	AAA	22	21	105
A6S-4-H2	DYKD	VTFTSAVFHENFYDWFVRQVS	AAA	20	19	104
A6S-4-D1	DYKD	SNPSRQDASVNFYDWFVREVA	AAA	22	22	103
A6S-4-H1	DYKD	IVAGARHSEVNFYDWFVIQVR	AAA	18	18	102
A6S-4-E2	DYKD	?DGQSVSSKGNFYDWFVQqMT	AAA	25	25	101
A6S-4-G1	DYKD	AELVGAGVRGNFYDWFVDQLV	AAA	16	16	101
A6S-4-G2	DHKD	SAGHHMPRESNFYDWFVDQVV	AAA	24	25	99
A6S-4-A1	DYKD	DSSRLWLGERNFYDWFVAqIS	AAA	12	17	68

FIGURE 28 (Cont.)

Name	Sequence	#Found	Ratio	IGF Inh.	GHR
H5:	LCQSLGVITYPGWLAGWCA	-	1.2	-	2.0
2C3:	VCQRLGGTFPGWLVGVCR	-	1.1	-	1.1
JBAS:	LCQSWGVRIGWLAGLCP	19	~24.0	~45%	1.2
E2A12:	LCQSLGFTDLWLACWFE	10	~17.5	~54	1.1
E1A11:	VCQSLGITDLGLCAGWGA	1	16.4	50	1.0
E4B10:	LCQSLGLTHPGFEAWLCA	5	~11.7	~50	1.2
E4C10:	LCQNLFGVTDPGCFYGWFA	1	9.9	~51	0.7
E4A9:	LCQSSGLSFLGCL-GWWA	14	~8.5	~65	1.0
JB36:	PCQRLGDTHLCWLAGWFA	6	~8.3	~65	1.1
E1F9:	PCQSLGLTCSGWFEWGGA	1	8.3	68	1.2
E4G7:	QWQSLGVTCPGSWAELCA	1	6.0	50	1.3
E4A11:	LWQSVGIKYPGGLAGWLA	1	5.8	67	1.4
E1B9:	LCQSLGVITYWEGLAWLCA	3	5.5	60	1.1
E4A12:	VCQSLGVECPGWFAWGA	3	~5.3	~55	1.2
E4F11:	LCQGWGIRIGWLVGRCM	1	2.7	58	1.1
E1D3:	LCQSLGVITYPGWLAGGCA	1	2.0*	-	1.0

FIGURE 29

Genomic rVab Library

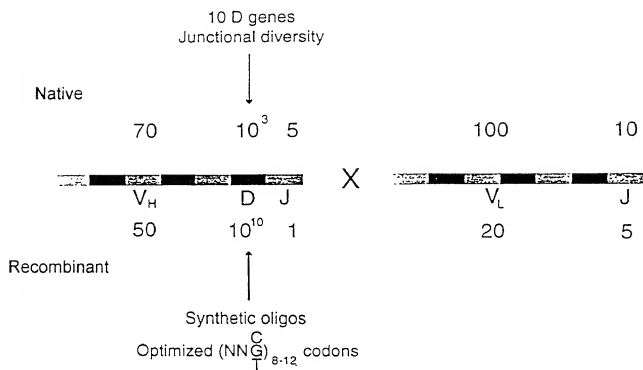


FIGURE 30

VH Gene Sequences

DP-1
DP-10
DP-12
DP-14
DP-15
DP-2
DP-21
DP-25
DP-29
DP-30
DP-31
DP-32
DP-33
DP-35
DP-38
DP-39
DP-40
DP-42
DP-44
DP-45
DP-46
DP-47
DP-5
DP-50
DP-51
DP-52
DP-53
DP-54
DP-59
DP-63
DP-66
DP-67
DP-68
DP-69
DP-7
DP-70
DP-71
DP-73
DP-74
DP-8
hv1263
VHD26

Lambda and Kappa Gene Sequences

DPK11
DPK15
DPK18
DPK2/L14+
DPK3/L11+
DPK4
DPK6
DPK8/Vd+
DPL23
HK101
L22+
L23/L23a
LFVK431
VA++

FIGURE 31

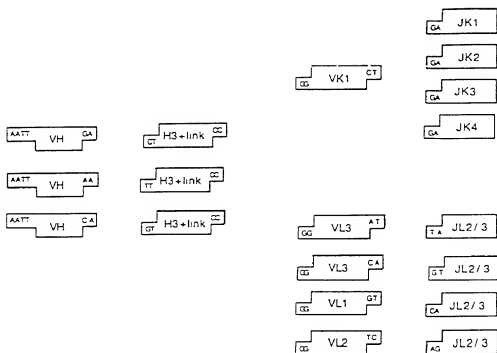


FIGURE 32

10	20	30	40	50	60	
1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	
GCCACGCGG	CCATGGCCCA	GGTGCAGCTG	GTTGAGTCTG	GCGGAGGCTT	GGTAAAGGCT	60
	Q	V Q L	V E S G	G G L	V K P	
G333GGTCC	TTAGACTCTC	CTGTGCAGCC	TCTG3MITCA	CTTTCAGTAA	CGCTCGGATG	120
G G S L	R L S	C A A	S G F T	F S N	A W M	
AGCTGGGTCC	GCCAGGCTCC	AGGGAAGGGG	CTGGAGTGGG	TTGGCGGTAT	TAAAGCAAA	180
S W V R	Q A P	G K G	L E W V	G R I	K S K	
ACTGATGGTG	GGACAACAGA	CTACGCTGCA	CCCGTGAAG	GCAGATTAC	CATCTCAAGA	240
T D G G	T T D	Y A A	P V K G	R F T	I S R	
GATGATTCAA	AAACACGGT	GTATCTGCAA	ATGAACAGCC	TGAAACCGA	GGACACAGCC	300
D D S K	N T L	Y L Q	M N S L	K T E	D T A	
GTTGATTACT	GTACCAAGT	TGGTGTGTCT	GCCGACCGTG	GGATGTGGGG	TCAAGGAACT	360
V Y Y C	T T V	A L S	A D R G	M W G	Q G T	
CTGCTACCG	TCTCTCAGG	TGGAGGCGGT	TCAGGCGGG	GTTGGCTCTG	CGGTGGCGGA	420
L V T V	S S G	G G G	S G G G	G S G	G G G	
TCGGATGTG	TGATGACTCA	GTCTCCACTC	TCCCTGCCCG	TCACCCCTTG	ACAGCCGCGC	480
S D V V	M T Q	S P L	S L P V	T L G	Q P A	
TCCATCTCT	GCAGGTCTAG	TCAAGCGCTC	GTATACAGTG	ATGGAACAC	CTACTTGAAT	540
S I S C	R S S	Q S L	V Y S D	G N T	Y L N	
TGGTTCAGC	AGAGCCGAG	CCAATCTCCA	AGGCGCTTAA	TTTATAGGT	TTCTAACCGG	600
W F Q Q	R P G	Q S P	R R L I	Y K V	S N R	
GACTCTGGGG	TOCCAGACAG	ATTCAAGGCG	AGTGGGTGAG	GCACGTGATT	CACACTGAAA	660
D S G V	P D R	F S G	S G S G	T D F	T L K	
ATCAGCGGG	TGGAGGCTGA	GGATGTGGG	GTTTATTACT	GCATGCAAGG	TACACTCTGG	720
I S R V	E A E	D V G	V Y Y C	M Q G	T H W	
CCTTACACTT	TTGGCCAGGG	GACCAAGCTG	GAGATCAAG	CGGCGCG		767
P Y T F	G Q G	T K L	E I K			

FIGURE 34

10	20	30	40	50	60	
1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	
GCCCAGCCGG	CCATGCCCCA	GATGCAGCTG	GTGGAGTCTG	GGGGAGGCTT	GCTAAAGCCT	60
	Q	M Q L	V E S G	G G L	V K P	
GGGGGTTCC	TTAGCTCTCT	CTGTGAGCC	TCTGGAATCA	CTTTCAGTAA	CGCCTGGATG	120
G G S L	R L S	C A A	S G F T	F S N	A W M	
AGCTGGGTCC	GCCAGGCTCC	AGGGAGGGGG	CTGGAGTGGG	TTGGCCGTAT	TAAAAACAAA	180
S W V R	Q A P	G K G	L E W V	G R I	K S K	
ACTGATGGTG	GGACACAGA	CTACCGTGCA	CCCGTGAAG	GCAGATTGAC	CATCTCAAGA	240
T D G G	T T D	Y A A	P V K G	R F T	I S R	
GATGATTCAA	AAAAACGGT	GTATCTGCAA	ATGACAGCC	TGAAAACCGA	GGACACAGCC	300
D D S K	N T L	Y L Q	M N S L	K T E	D T A	
GTGTATTACT	GTACCACATG	GGGCTCCGTC	GACACGGACA	ACTACGCCAG	GTTTTGGGGT	360
V Y Y C	T T W	G S V	D T D N	Y A R	F W G	
CAAGGAATC	TGTACACGGT	CTCTCAGGT	GGAGCGGGTT	CAGCGGGAGG	TGGCTCTGGC	420
Q G T L	V T V	S S G	G G G S	G G G	G S G	
GGTGGGGAT	CCGACATCCA	GATGACCCAG	TCTCCATCT	CCCTGTCTGC	ATCTGTAGGA	480
G G G S	D I Q	M T Q	S P S S	L S A	S V G	
GACAGAGTCA	CCATCACTTG	CCGGGGAGT	CAGGGCATTA	GCATTTATTT	AGCCTGTGAT	540
D R V T	I T C	R A S	Q G I S	N Y L	A W Y	
CAGCAGAAC	CAGGGAAAGT	TCTTAAGCTC	CTGATCTATG	CTGCATCCAC	TTTTCGAATCA	600
Q Q K P	G K V	P K L	L I Y A	A S T	L Q S	
GGGTGCCAT	CTGGTTCAG	TGGCAGTGA	TCTGGACAG	ATTTCATCT	CACCATCAGC	660
G V P S	R F S	G S G	S G T D	F T L	T I S	
AGCCTGCAGC	CTGAAGATGT	TGCAACTTAT	TACTGTCAAA	AGTATAACAG	TGCCCCCTCT	720
S L Q P	E D V	A T Y	Y C Q K	Y N S	A P L	
ACTTTGGGCG	GAGGGACCAA	GGTGGAGATC	AAAGCGGGCG	C		761
T F G G	G T K	V E I	K			

FIGURE 35

10	20	30	40	50	60	
1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	
GCCACGCGG	CCATGGCCCA	GATCCAGCTG	GTCGAGTCTG	GCGGAGGCTT	GCTAAAGGCT	60
	Q	M Q L	V E S G	G G L	V K P	
GCGGGTCC	TTAGACTCTC	CTGTGCAGCC	TCGTGATTC	CTTTCAGTAA	CGCGTGGATG	120
G G S L	R L S	C A A	S G F T	F S N	A W M	
AGCTGGGTCC	GCCAGGCTCC	AGCGAAGGGG	CTGGAGTGGG	TTGGCCGTAT	TAAAGCAAA	180
S W V R	Q A P	G K G	L E W V	G R I	K S K	
ACTGATGGTG	GGACACAGA	CTACGCTGCA	CCCGTGAAG	GCAGATTAC	CATCTCAAGA	240
T D G G	T T D	Y A A	P V K G	R F T	I S R	
GATGATTCAA	AAAAACGGCT	GTATCTGCAA	ATGAACAGCC	TGAAAACGA	GGACACAGCC	300
D D S K	N T L	Y L Q	M N S L	K T E	D T A	
GTGTATTACT	GTACCACACC	GCGCTGGTAT	GCGCCCGAGG	ATAAGTGGGG	TCAAGGAATC	360
V Y Y C	T T P	G W Y	G A E D	K W G	Q G T	
CTGTCAAGG	TCTCTCAGG	TGGAGCGGCT	TCAGGCGGAG	GTCGCTCTGG	GCGTGGCGGA	420
L V T V	S S G	G G G	S G G G	G S G	G G G	
TCCGACATCC	AGATGAACCA	GTCTCCATCC	TCCGTGCTG	CATCTGTAGG	AGACAGAGTC	480
S D I Q	M T Q	S P S	S L S A	S V G	D R V	
ACCATCACTT	GCGCGGGGAG	TCAGGCGCAT	AGCAATTATT	TAGCGTGGTA	TCAGCAGAAA	540
T I T C	R A S	Q G I	S N Y L	A W Y	Q Q K	
CCAGGGAAG	TTCCTAAGCT	CCTGATCTAT	GCTGCATCCA	CTTTGCAATC	AGGGGTCCCA	600
P G K V	P K L	L I Y	A A S T	L Q S	G V P	
TCGTGGTTC	GTCGAGTGG	ATCTGGGACA	GATTTCACTC	TCACCATCAG	CAGCGTCAG	660
S R F S	G S G	S G T	D F T L	T I S	S L Q	
CCTGAAGATG	TTCGAACCTA	TTACTGTCAA	AAGTATACAA	GTCGCCCTTT	CACITTCGGC	720
P E D V	A T Y	Y C Q	K Y N S	A P F	T F G	
CCTGGGACCA	AAGTGGATAT	CAAAAGCGGC	GC			752
P G T K	V D I	K				

FIGURE 36

10	20	30	40	50	60	
1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	
GCCGAGCGGG	CCATGGCCCA	GGTGCAGCTG	GTCGAGTCTG	GCGGAGGCTT	GGTACAGCCT	60
	Q	V Q L	V E S G	G G L	V Q P	
GXXGATCUC	TGAGACTCTC	CITGTGAGCC	TCTGSAITCA	CCATCAGTAA	CAGTGACATG	120
G G S L	R L S	C A A	S G F T	F S N	S D M	
AACGGGTC	ATCAGGCTCC	AGGAAGGGG	CTGAGTGGG	TATCGGGTGT	TAGTGGAT	180
N W V H	Q A P	G K G	L E W V	S G V	S W N	
GCCAGTAGGA	CGCACTATGC	AGACTCTGTG	AAGGCCGAT	TGATCATCTC	CAGAGCAAT	240
G S R T	H Y A	D S V	K G R F	I I S	R D N	
TCCAGGACA	CCCTGTATCT	GCAACGAAT	AGCTGAGGG	CCGAGGACAC	GCTGTGTAT	300
S R N T	L Y L	Q T N	S L R A	E D T	A V Y	
TACTGTGTGA	GAACGATGG	CGATGTGTAC	GCGGCTGGG	GTCAGGAC	TCTGTGACC	360
Y C V R	T D G	E W Y	G A W G	Q G T	L V T	
GTCCTCTCAG	GTCGAGCGG	TTCAGGCGGA	GCTGCTCTG	GCGGTGGGG	ATCGGCCATC	420
V S S G	G G G	S G G	G G S G	G G G	S A I	
CAGATGACC	AGTCTGCATC	CTCCCTGTCT	GCATCTGTAG	GAGACAGAT	CACCATCACT	480
Q M T Q	S P S	S L S	A S V G	D R V	T I T	
TCCCGGGCA	GTCAGGCGAT	TAGAAATGAT	TTAGGCTGCT	ATCAGCGAA	ACCAGGGAA	540
C R A S	Q G I	R N D	L G W Y	Q Q K	P G K	
GCCCTTAGC	TCCGATCTA	TGCTGCATCC	AGTTTACAAA	GTCGGGTCCC	ATCAGGTTC	600
A P K L	R I Y	A A S	S L Q S	G V P	S R F	
AGCGGCGAGT	GATCTGGCAC	AGATTTCACT	CTCACCATCA	GCAGCTGCA	GCTGAAGAT	660
S G S G	S G T	D F T	L T I S	S L Q	P E D	
TTTGCAACTT	ATTACTGTCT	ACAAGATTAC	AATTACCCCTC	TCACTTTCGG	CGGAGGGACC	720
F A T Y	Y C L	Q D Y	N Y P L	T F G	G G T	
AGGTGGGGA	TCAAGGGGC	CGC				743
K V E I K						

FIGURE 37

	10	20	30	40	50	60	
	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	
	GCCCAGCCGG	CCATGGCCCC	GATCCAGCTG	GTCGAGTCTG	GCGGAGGCTT	GGTACAGGCT	60
		Q	M Q L	V Q S G	G G L	V Q P	
	GCGGGTACC	TGAGACTCTC	CTGTGACGAC	TCTGGATTCA	CCCTCAGTAG	CTATGCTATG	120
	G G S L	R L S	C A G	S G F T	F S S	Y A M	
	CAGTGGGTTC	GCCAGGCTCC	AGGAAAGGT	CTGGAGTGGC	TATCAGCTAT	TGTACTGTGT	180
	H W V R	Q A P	G K G	L E W V	S A I	G T G	
	GGTGGACAT	ACTATGAGA	CTCCGTGAAG	GCCGATTCA	CCATCTCCAG	AGACAATGCC	240
	G G T Y	Y A D	S V K	G R F T	I S R	D N A	
	AAGAATCCT	TGTATCTTCA	AATGAACAGC	CTGAGAGCCG	AGGACATGGC	TGTGTATTAC	300
	K N S L	Y L Q	M N S	L R A E	D M A	V Y Y	
	TGTGGAAGAT	GCGGGCAGCT	GCGCTTGTGG	GTTGGGAGCG	TGTATTGGGG	TCAAGGAAT	360
	C A R W	G H V	G L W	V A D V	Y W G	Q G T	
	CTGGTACCG	TCTCTCAGG	TGGAGGCGGT	TCAGGCGGAG	GTCGCTCTGG	CGGTGGCGGA	420
	L V T V	S S G	G G G	S G G G	G S G	G G G	
	TCCGACATCC	AGATGAACCA	GCTCCATCC	TCCCTGTCTG	CATCTGTAGG	AGACAGATTC	480
	S D I Q	M T Q	S P S	S L S A	S V G	D R V	
	AGCATCATTT	GCCGGGCGAG	TCAGGGCAAT	AGCAATATTT	TAGCCTGGTA	TCAGCAGAAA	540
	T I T C	R A S	Q G I	S N Y L	A W Y	Q Q K	
	CCAGGGAAG	TTCTTAAGCT	CCGTATCTAT	GCTGCATCCA	CTTTGCAATC	AGGGGTCCCA	600
	P G K V	P K L	L I Y	A A S T	L Q S	G V P	
	TCTGGTTCA	GCGGAGTGG	ATCTGGGACA	GATTCTACTC	TCACCATCAG	CAGCCTCCAG	660
	S R F S	G S G	S G T	D F T L	T I S	S L Q	
	CCGGAAGAT	TTGCACTTAA	TTACTGTCAA	AAGTATAACA	GTCGCCCTTA	CACCTTTGGC	720
	P E D V	A T Y	Y C Q	K Y N S	A P Y	T F G	
	CAGGGGACA	AGCTGGAGAT	CAAGGGGGCC	GC			752
	Q G T K	L E I	K				

FIGURE 38

10	20	30	40	50	60	
1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	
GCCAGCGCG	CCATGCCCCA	GATCCAGCTG	GTCAGTCTG	GCGAGGCTT	GATACGCTT	60
	Q	M Q L	V Q S G	G G L	V Q P	
GCGCGTCC	TGAGCTCTC	CTGTCCAGGC	TCTGCAITCA	CCITCAGTAG	CTATGCTAIG	120
G G S L	R L S	C A G	S G F T	F S S	Y A M	
CACTGCGTTC	GCCAGGCTCC	AGGAAAAGGT	CTGGAGTGGG	TATCAGCTAT	TGGTACTGTT	180
H W V R	Q A P	G K G	L E W V	S A I	G T G	
GCTGCGACAT	ACTATGCCA	CTCCGTGAAG	GCGCGAITCA	CCATCTCCAG	AGACATGCC	240
G G T Y	Y A D	S V K	G R F T	I S R	D N A	
AAGACTCTCT	TGTATCTTCA	AATGAACAGC	CTGAGAGCGG	AGGACATGGC	TGTGTATTAC	300
K N S L	Y L Q	M N S	L R A E	D M A	V Y Y	
TGTGCAAGAG	AGGCGAGCT	CGGGGTGACC	TCTTCTCTGG	GTCAGGAAAC	TCTGTCTACC	360
C A R E	G E L	G V T	S F W G	Q G T	L V T	
GTCCTCTCAG	GTGAGCGCG	TTCAAGCGGA	GTTGCTCTG	GCGGTGCGG	ATCCGACATC	420
V S S G	G G G	S G G	G G S G	G G G	S D I	
CAGATGACCC	AGTCTGCATC	CTCCCTGTCT	GCATCTGTAG	GAGACAGAGT	CACATCACT	480
Q M T Q	S P S	S L S	A S V G	D R V	T I T	
TGCGCGCGA	GTCAGGCGAT	TAGCAATAT	TTAGGCTGCT	ATCAGCAGAA	ACCAGCGAAA	540
C R A S	Q G I	S N Y	L A W Y	Q Q K	P G K	
GTTCTTAAAC	TCTGTATCTA	TGCTGCATCC	ACTTTGCAAT	CAGGGTTC	ATCTCGGTTT	600
V P K L	L I Y	A A S	T L Q S	G V P	S R F	
AGTGCAGTG	GATCTGGGAC	AGATTTCAT	CTCACCATCA	CCAGGCTGCA	GCTGGAAGAT	660
S G S G	S G T	D F T	L T I S	S L Q	P E D	
GTTGCACTT	ATTACITGCA	AAAGTATAAC	AGTGCCCCCT	GGAGTTCGG	CCAAGGAC	720
V A T Y	Y C Q	K Y N	S A P W	T F G	Q G T	
AAGGTGAAA	TCAAAGCGGC	CGC				743
K V E I	K					

FIGURE 39

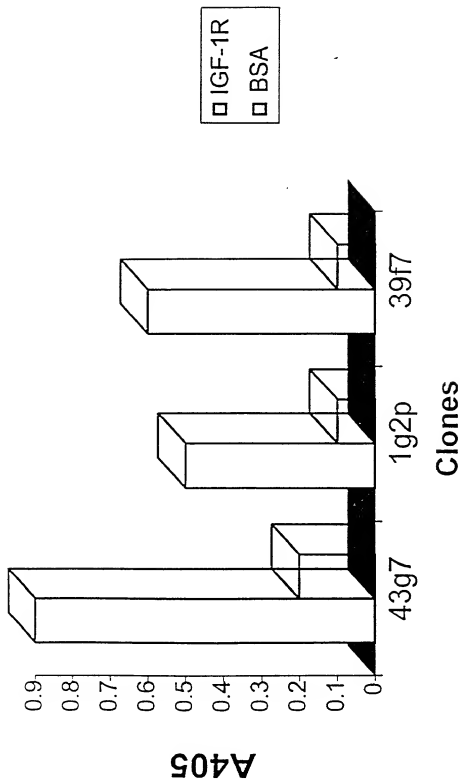


FIGURE 40

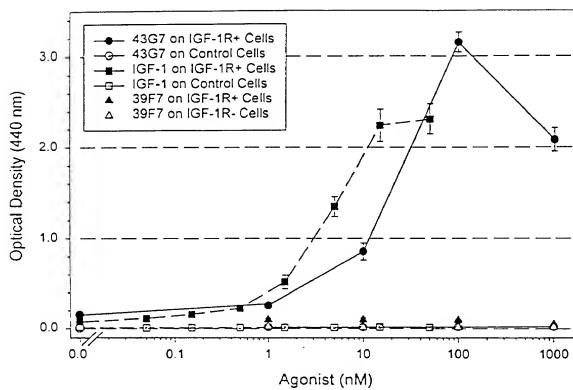


FIGURE 41

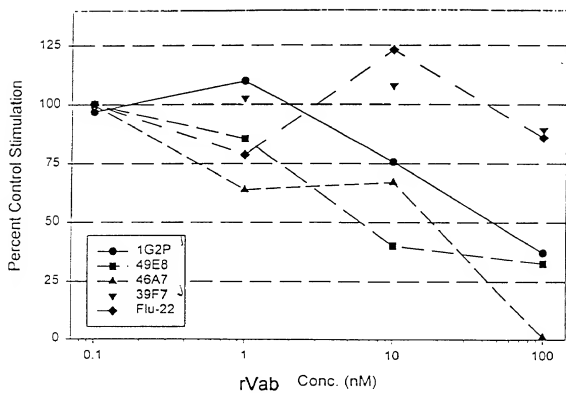


FIGURE 42

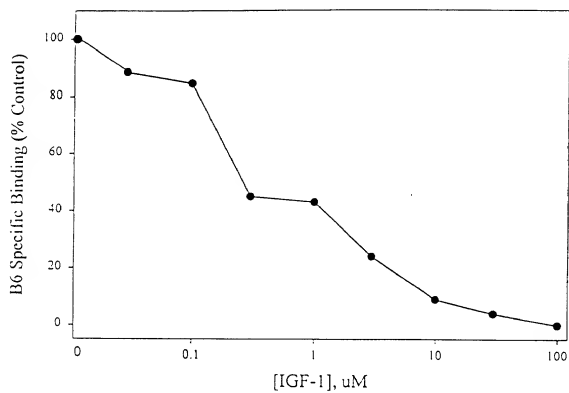


FIGURE 43

rVab 43G7 Specific Binding (% Control)

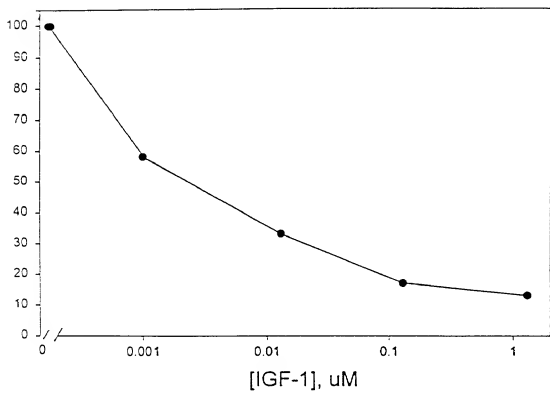


FIGURE 44

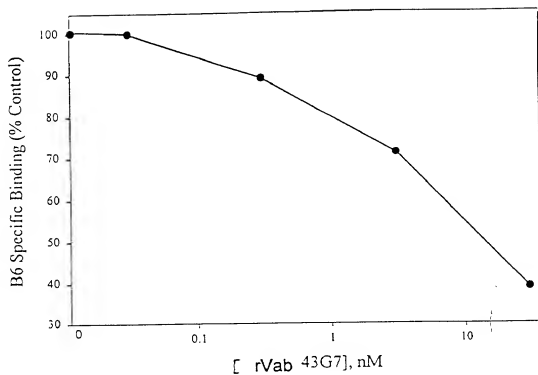


FIGURE 45

FIGURE 46A

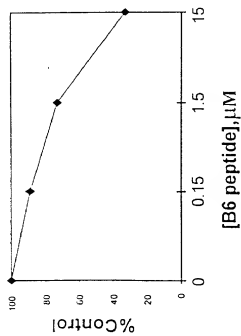


FIGURE 46B

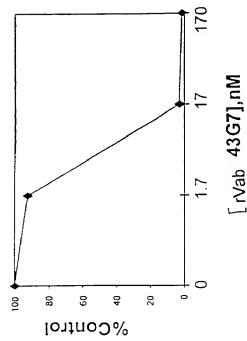


FIGURE 46C

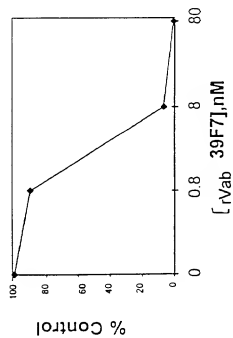
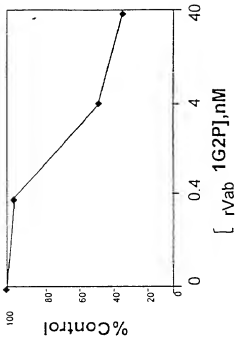


FIGURE 46D



Group 1: Formula 1 Motif

			Found	IR	Target	IGF
20D3*	IGGOGHODGNFYDFVEALA		18	+		+++
20F1	VFWNCRSQQLDFYWFQRA		16	+		+++
G3	RGGTFYWFESALRKHGAG		8	+		+++
20H1	RVAGAIAPGLVSNKQDGLFYWFRE		5	+		+++
20D1*	VLQARIHGCDVSVDIFYWFA		4	+		+++
D2	DPERMQSDVGEYWFERRAVG		3	+		+++
B8*	WSALLSVMDTGFYAFWDDAV		2	++		+++
C4	DIGSDGHRWRWDSFYWFEM		2	+		+++
A8	IGGSFVEFYGFENDQV		2	+		+++
E7*	GHSWALVRHVRDLFYWFEDL		1	++		+++
C8*	LPAGGAGGFAYRGFYWFES		1	+		+++
1R	RDKPTDQEQNSFYWFRII		1	+		+++
E2	SRDQTNETNSAGFYWFEEER		1	+		+++
B12	GNYFRWFIEALVCSERVPDV		1	+		++
D10-2	RIGGGWARSEGEFYWFVREL		1	+		++
G8	RMEFYWFNSQMGAPTEGSA		1	+		++
13	HEAFYDFWESALVDGGYELMG		1	+		++
3G11	FYGVESRQLSLTPRDDMGILP		1	+		++
F4	GVGTLTNSSDAFYTFVF		1	+		++
E7-2	LGTSAGQGVGHAFYQWFQS		1	+		+
40G11	<---ETLSHYVVTG-----		3	+		+++
40I2	TRDNHHYVWQRRRVINCVRQWYISDRYNDGSAFYWFID		2	+		++
40B12	RMGLQALAHYRKGA-----GPIFLSSGSVIKSGEDPFYAFERLQ		1	+		++

FIGURE 47

FIGURE 49A

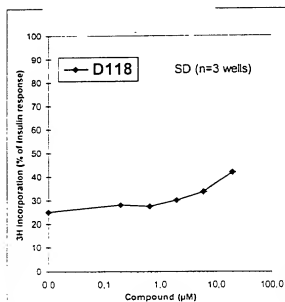


FIGURE 49B

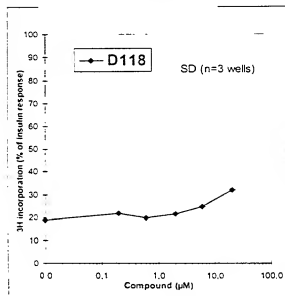
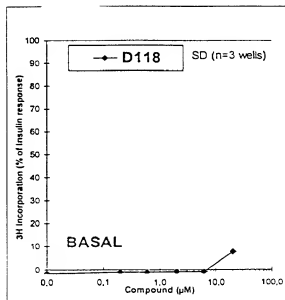


FIGURE 49C

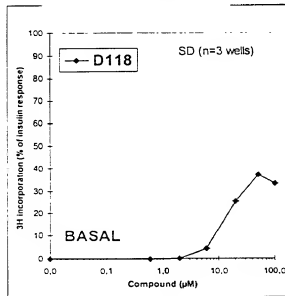


FIGURE 49D

FIGURE 50A

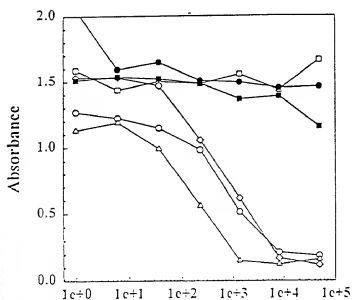


FIGURE 50B

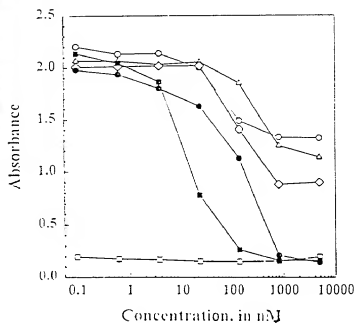
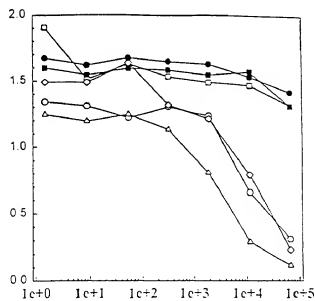


FIGURE 50C

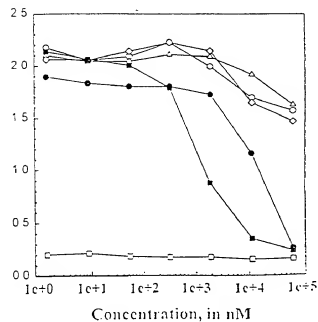


FIGURE 50D

FIGURE 51A

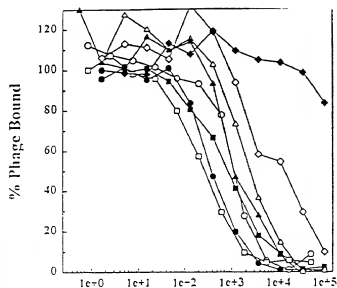


FIGURE 51B

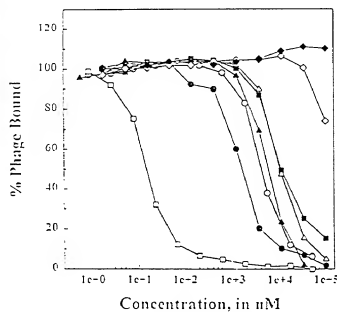
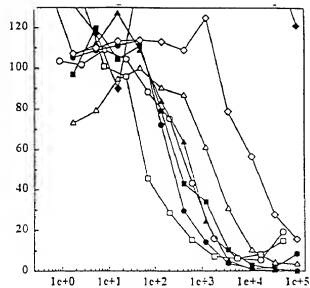


FIGURE 51C

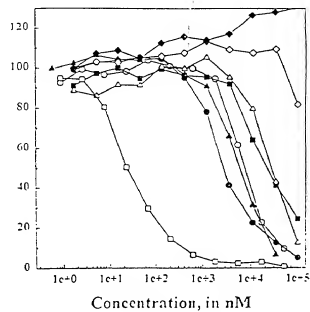


FIGURE 51D

FIGURE 52A

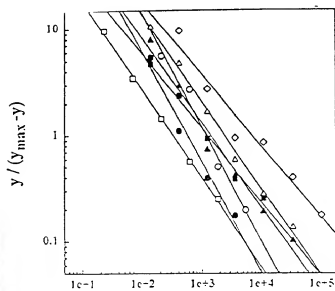


FIGURE 52B

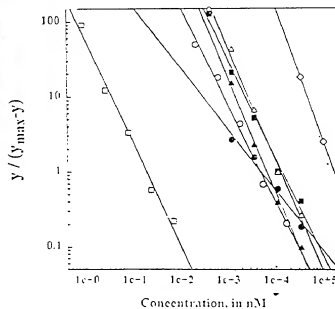
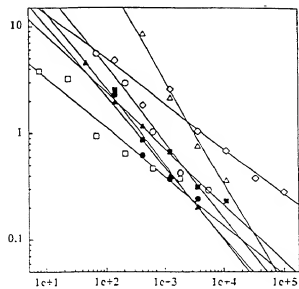


FIGURE 52C

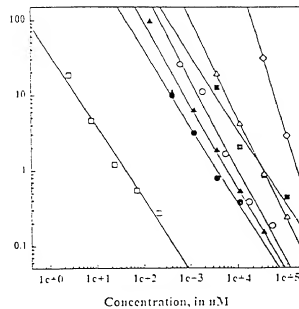


FIGURE 52D

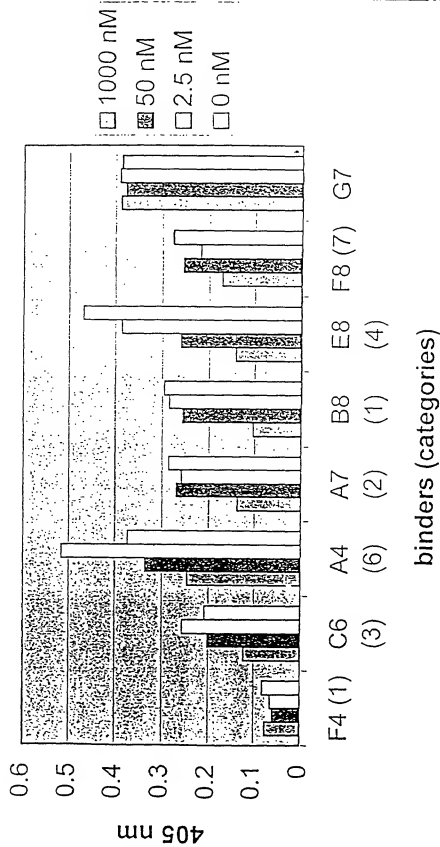


FIGURE 53

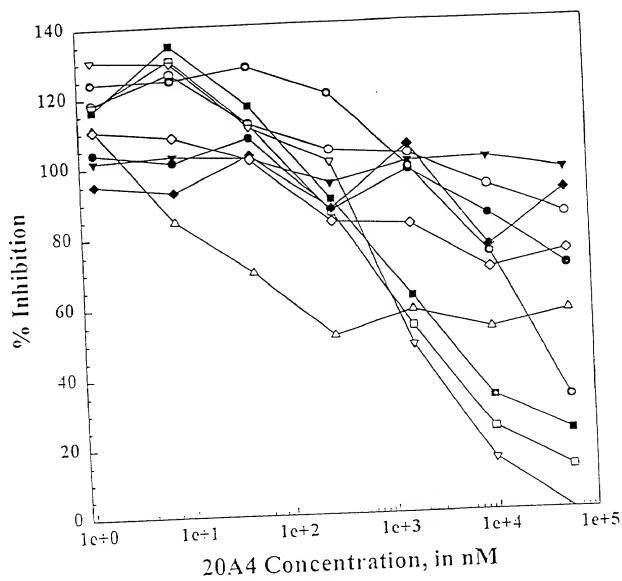


FIGURE 54

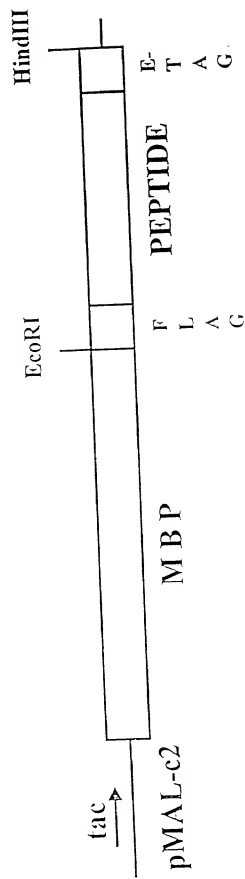


FIGURE 55

FIGURE 56B

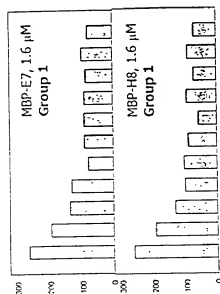


FIGURE 56A

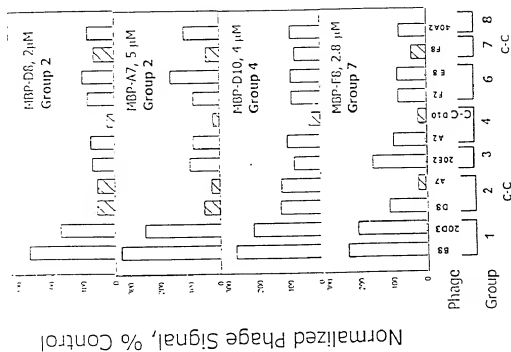
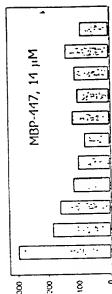


FIGURE 56C



10	20	30	40	50	60														
1234567890	1234567890	1234567890	1234567890	1234567890	1234567890														
GCCCAGCCCG	CCATGCGCCG	G3TGCAGCTG	GTGGAGTCTG	G3GGAGGCTT	G3TAAAGCCT	60													
	E	V	Q	L	V	E	S	G	G	G	L	V	K	P					
G3G3GGTCCC	TTAGACTCTC	CTGTGCAGCC	TCTGGATTCA	CTTTCACTAA	CGCGTGGATG	120													
G	G	S	L	R	L	S	C	A	A	S	G	F	T	F	S	N	A	W	M
AGCTGGGTCC	GCGAGGCTCC	AGCGAAGGGG	CTGGAGTGGG	TTGGCGGTAT	TAAAGCAAA	180													
S	W	V	R	Q	A	P	G	K	G	L	E	W	V	G	R	I	K	S	K
ACTGATGCTG	GGCAACAGA	CTACGCTGCA	CCCGTGAAG	GCGATTTCAC	CATCTCAAGA	240													
T	D	G	G	T	T	D	Y	A	A	P	V	K	G	R	F	T	I	S	R
GATGATTCAA	AAACACGGT	GTATCTGCA	ATGACAGCC	TGAAACCGA	GGACACAGCC	300													
D	D	S	K	N	T	L	Y	L	Q	M	N	S	L	K	T	E	D	T	A
GTGATTACT	GTACCACTA	CGCGAGCGTT	TACGACCGCG	ATTACGATGG	GCGCTGGGGT	360													
V	Y	Y	C	T	T	Y	G	D	V	Y	D	R	D	Y	D	G	R	W	G
CAGGAACTC	TGGTACCGT	CTCTCTAGGT	GAGGCGGGTT	CAGGCGAGG	TGGTCTGGC	420													
Q	G	T	L	V	T	V	S	S	G	G	G	G	S	G	G	G	G	S	G
GTTGGCGGAT	CGGACATCCA	GATGACCCAG	TCTCATCTCT	CGTGTCTGCG	ATCTGTAGCA	480													
G	G	G	S	D	I	Q	K	T	Q	S	P	S	S	L	S	A	S	V	G
GACAGATCA	CCATCATCTG	CGGGCGAGT	CAGGGCATTA	GCAATTATTT	AGCGTGGTAT	540													
D	R	V	T	I	T	C	R	A	S	Q	G	I	S	H	Y	L	A	U	Y
CAGGAGAAC	CAGGGAAGT	TCTTAGGTC	CTGTCTATG	CTCTCTCAC	TTTGTATCA	600													
Q	Q	K	P	G	K	V	P	K	L	L	I	Y	A	A	S	T	L	Q	S
G3G3TCCCT	CTCGTCTAG	TGGGATGGA	TCTGGGACAG	ATTCTACTCT	CACATCAGC	660													
G	V	P	S	R	F	S	G	S	G	S	G	T	D	F	T	L	T	I	S
AGGTCGAGC	CTGAGATGT	TGCACTTAT	TACTGTCAA	AGTATACAG	TGCGGCTTTC	720													
S	L	Q	P	E	D	V	A	T	Y	Y	C	Q	K	Y	H	S	A	P	F
ACTTTCGTC	CTGGAACCA	AGTGAATAT	AAACGCGCG	C		761													
T	F	G	P	G	T	H	V	D	I	K									

FIGURE 57

10	20	30	40	50	60	
1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	
GCCCATCCGG	CCATGGCCCG	GATCGAGCTG	TTGGAGTCTG	GCGGAGGCTT	GCTAAGGCTT	60
	E V Q L	L E S G	G G L	V K P		
GCGGGTCCC	TTAGATCTC	CTGTGCAGCC	TCTGGATTC	CTTTCACTAA	CGCTGGATG	120
G G S L	R L S	C A A	S G F T	F S N	A W H	
AGTGGGTTCC	GCCAGGCTCC	AGGAGGCGG	CTGGAGTGG	TTGGCGGTAT	TAAAGCAAA	180
S W V R	Q A P	G K G	L E W V	G R I	K S K	
ACTGATGGTG	GACACACAG	CTAGGTCGA	CCGTTGAAG	GCGATTTAC	CATCTCAGA	240
T D G G	T T D	Y A A	P V K G	R F T	I S R	
GATGATTCGA	AAACACGCT	GATCTGCA	ATGACAGCC	TGAAACCGA	GACACAGCC	300
D D S K	N T L	Y L Q	M N S L	K T E	D T A	
GCTGATTAAT	GTAACACATA	CGATTAGTG	GCGGCTCTG	TGGTCTGGGG	TCAAGGACT	360
V Y Y C	T T Y	D . W	G V L V	V W G	Q G T	
CTGGTCAAG	TCTCTCAGG	TGGAGGCTT	TCAAGGCGG	GCTGCTCTG	GCTGAGCGA	420
L V T V	S S G	G G G	S G G G	G S G	G G G	
TCCAGCTCC	AGTTGACCA	GCTCCATCC	TTCTGCTCTG	CATCTGTAGG	AGACAGCTC	480
S D I Q	L T Q	S P S	F L S A	S V G	D R V	
AGCATCACTT	GCGGGGCGG	TCAAGGCTT	AGCATTAAT	TAGCTGCTA	TCAAGGAAA	540
T I T C	R A S	Q G I	S S Y L	A W Y	Q Q H	
CGAGGGAAG	CCCTAAGCT	CTGATCTAT	GCTGATCCA	CTTTGCAAG	TGGGTTCCCA	600
P G K A	P K L	L I Y	A A S T	L Q S	G V P	
TCAAGTTCA	GCGGAGTGG	ATCTGAGCA	GATTTCACTC	TCAACATCG	CAGCTGCGG	660
S R F S	G S G	S G T	D F T L	T I S	S L Q	
CTGAGGAT	TTCAGCTTA	TTCATCTCA	CAGCTTAATA	GTTACCTTTT	CATTTGCGG	720
P E D F	A T Y	Y C Q	Q L N S	Y P F	T F G	
CTGAGGATCA	AGTGGATAT	CAAGGCGCC	GCAAGTCC	-		758
P G T N	V D I K					

FIGURE 58

	CLONES	VHCDR3	
	%Inhibition Activity		
	118:	PFFV	FYRGQDT 54%
?	Insul18:	FVNQHLCGSHLVEAFLY LVCGERGEFFYTPKI	
	12H10:	C WYNYA G RG T	42%
Ang?	13-e-4:	VQANDGL G RES	52%
?	13h9:	GGL G RRDWL	30%
?	24:	GGRR H RLG	
	InsulinA	CTHEQCGTSTICSLYQ LENYQN	
	11a6:	ENYGNSE	32%
?		GDQELQNY	None
	N/A		

FIGURE 59

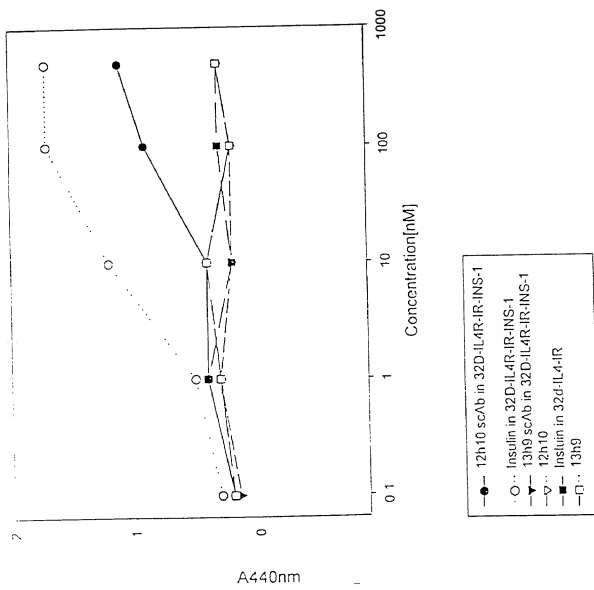


FIGURE 60

Figure 1 is a line graph showing the relationship between KFU (x-axis) and Insulin concentration in μM (y-axis). The x-axis ranges from 0 to 50, and the y-axis is a logarithmic scale ranging from 0.01 to 100. The data points are connected by a solid line, showing a sharp increase in KFU at low insulin concentrations, followed by a plateau and then a decrease at higher concentrations.

Insulin [μM]	KFU
0.01	~45
0.1	~48
1	~48
10	~32
30	~25
100	~15
300	~12
1000	~8

FIGURE 61

IGF [uM]	KFU
1.5	47.5
2.0	47.5
3.0	46.0
5.0	42.0
10.0	38.0
20.0	34.0
40.0	30.0
80.0	26.0

FIGURE 62

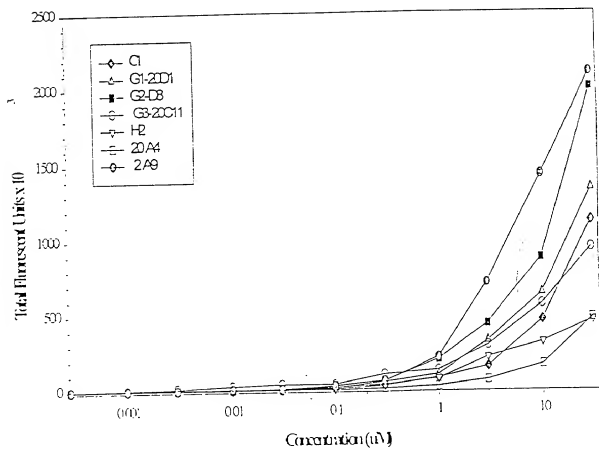


FIGURE 63

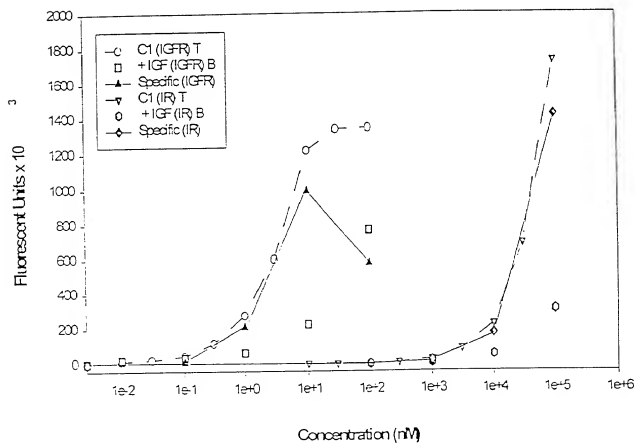


FIGURE 64

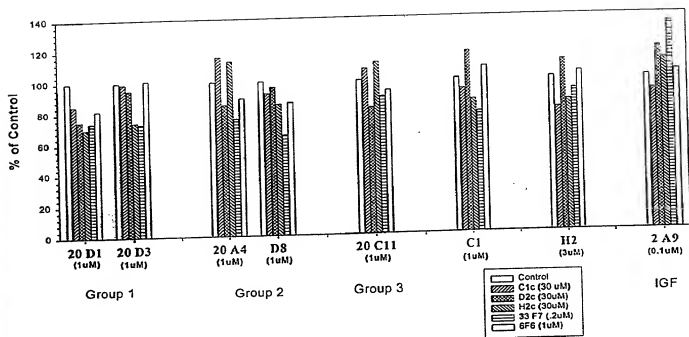


FIGURE 65

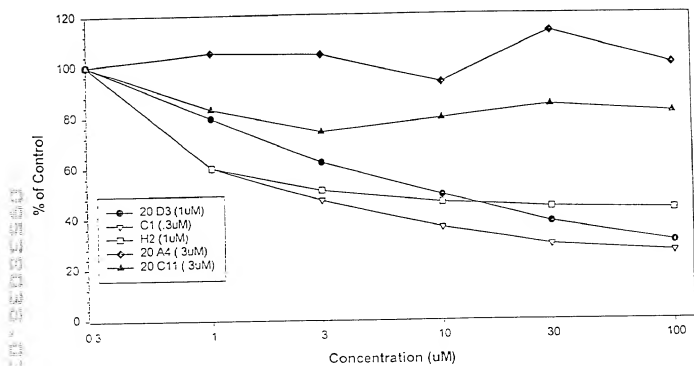


FIGURE 66

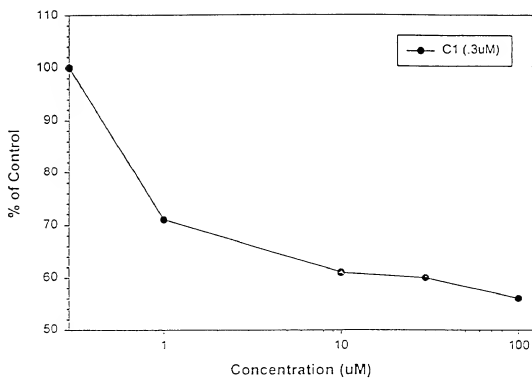


FIGURE 67

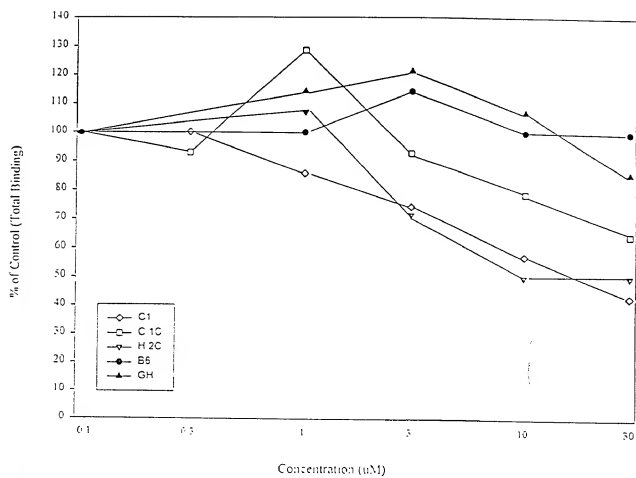


FIGURE 68

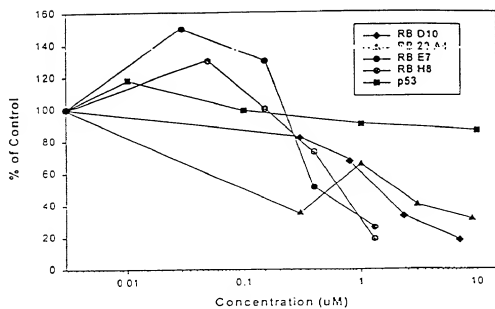


FIGURE 69

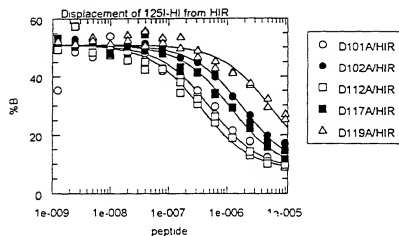


FIGURE 70A

D990114A

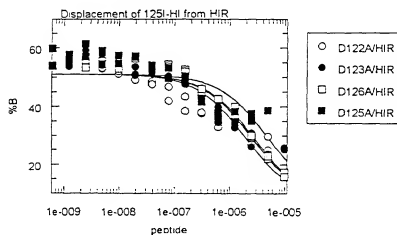


FIGURE 70B

D990118A

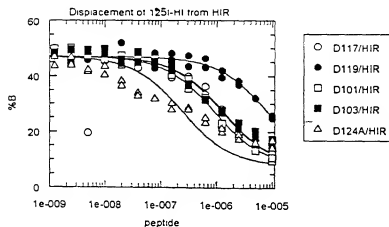


FIGURE 70C

D990126A

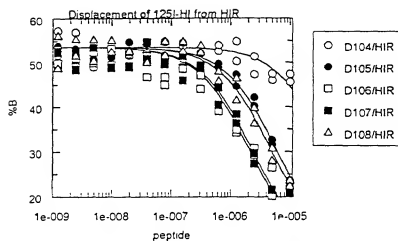


FIGURE 70D

D990129A

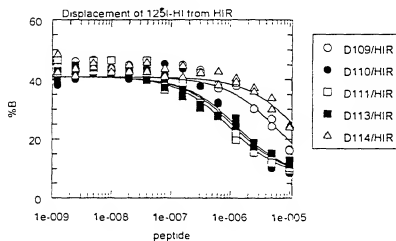


FIGURE 70E

D990202A

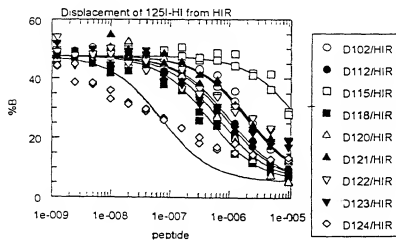


FIGURE 70F

D990205A

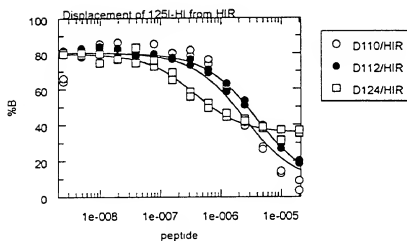


FIGURE 70G

D990217A

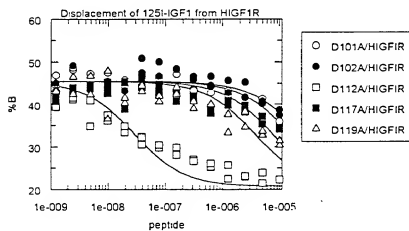


FIGURE 70H

D990114A

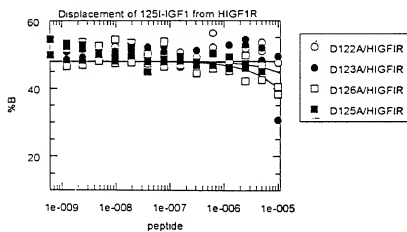


FIGURE 70I

1

D990118A

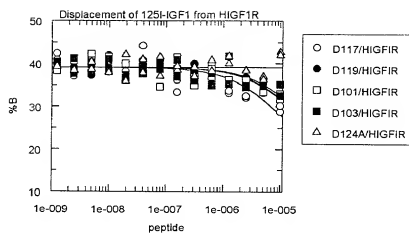


FIGURE 70J

D990126A

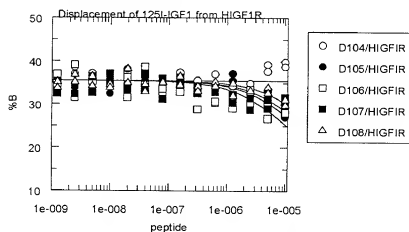


FIGURE 70K

D990129A

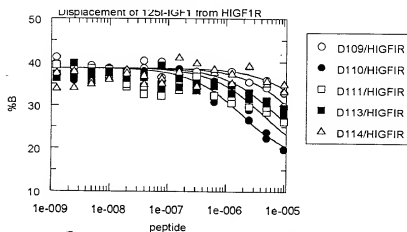


FIGURE 70L

D990202A

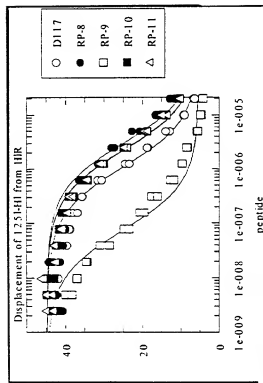


FIGURE 70M

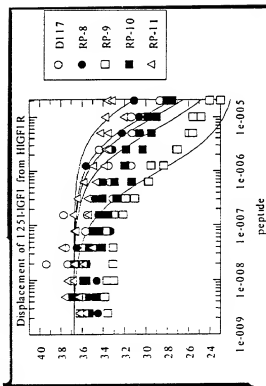


FIGURE 70N

FIGURE 71A

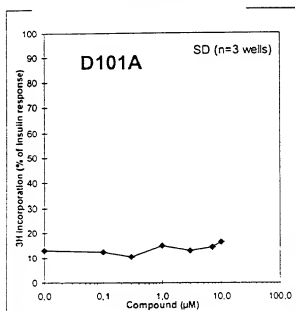


FIGURE 71B

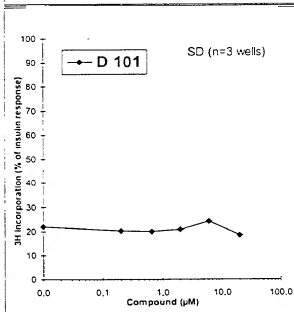
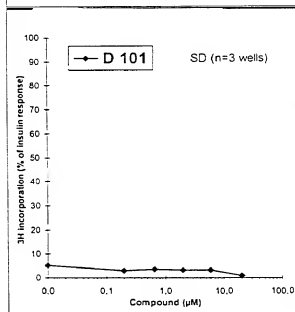
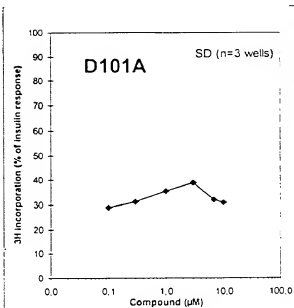


FIGURE 71C

FIGURE 71D

FIGURE 71E

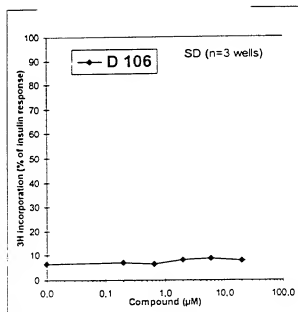


FIGURE 71F

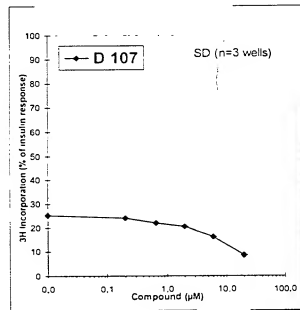
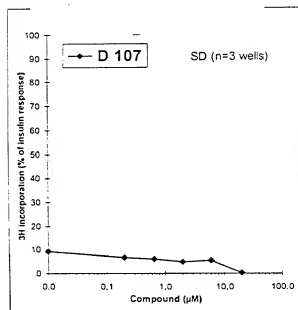
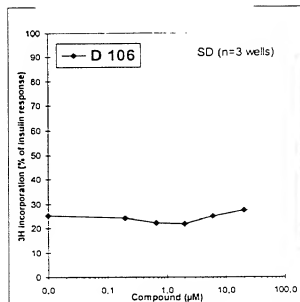


FIGURE 71G

FIGURE 71H

FIGURE 71I

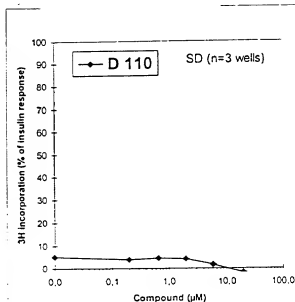


FIGURE 71J

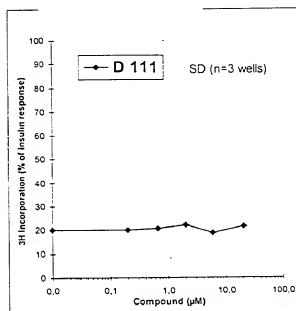
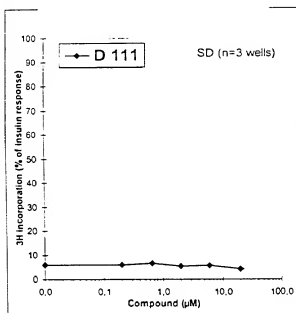
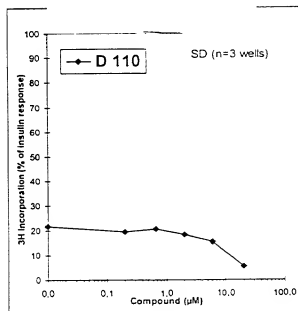


FIGURE 71K

FIGURE 71L

FIGURE 71M

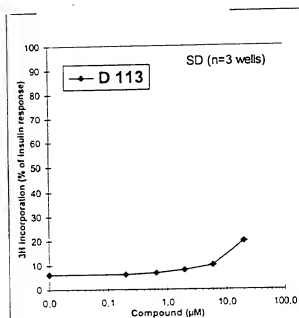


FIGURE 71N

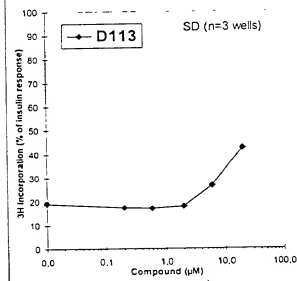
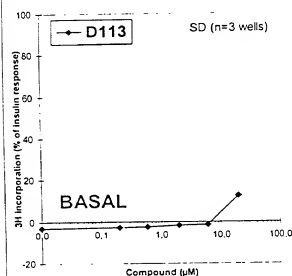
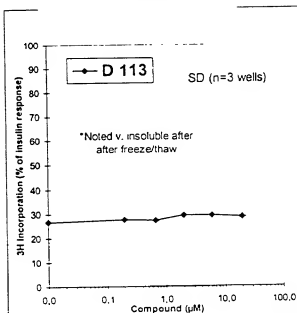


FIGURE 71O

FIGURE 71P

FIGURE 71Q

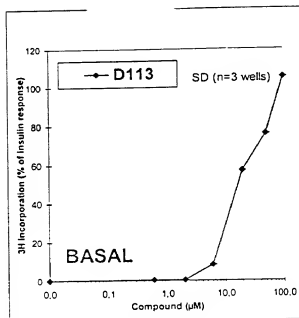


FIGURE 71R

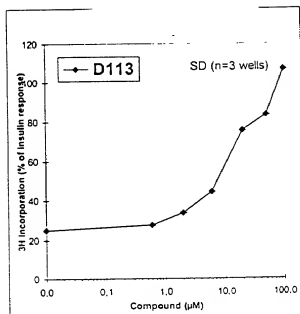


FIGURE 71S

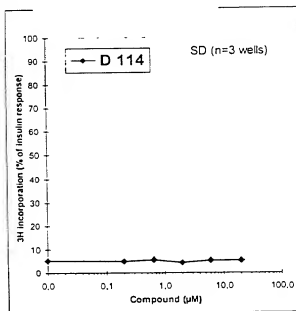


FIGURE 71T

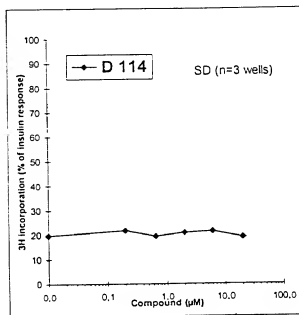


FIGURE 71U

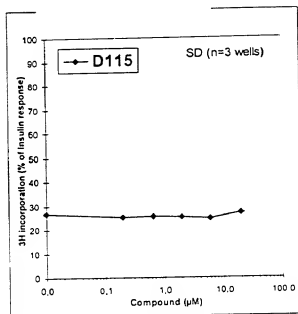


FIGURE 71V

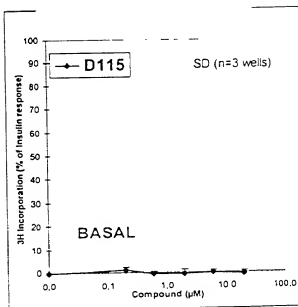


FIGURE 71W

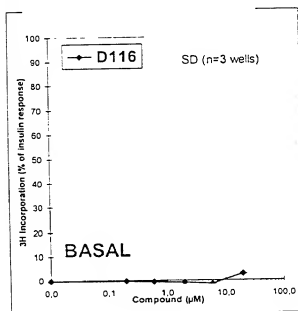


FIGURE 71X

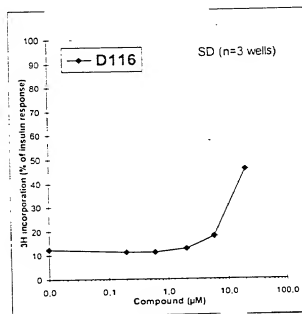


FIGURE 71Y

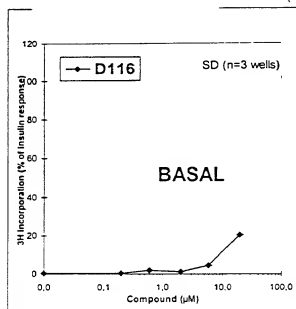


FIGURE 71Z

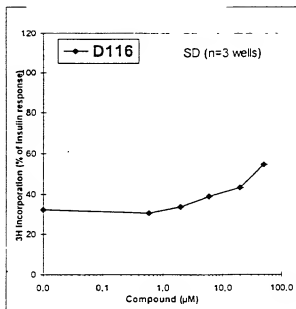


FIGURE 71A2

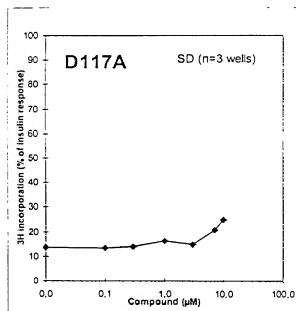


FIGURE 71B2

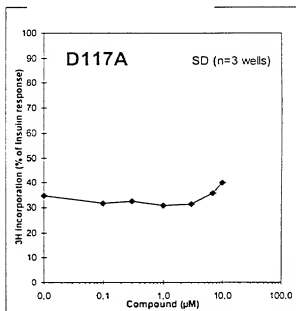


FIGURE 71C2

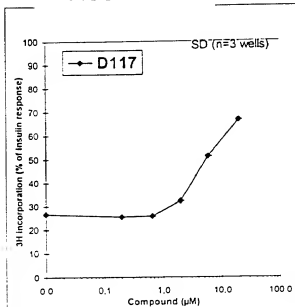


FIGURE 71D2

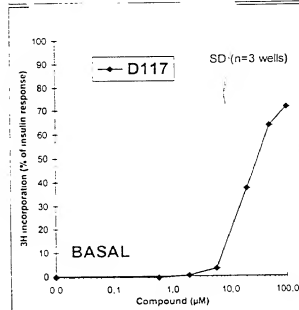
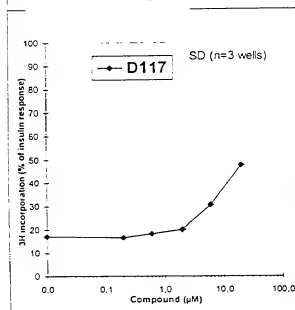
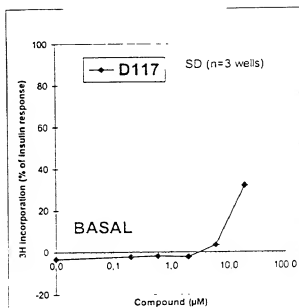


FIGURE 71E2

FIGURE 71F2

FIGURE 71G2

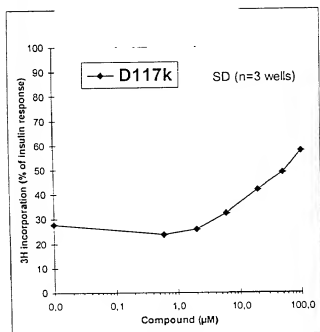
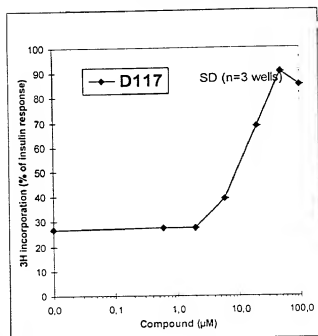


FIGURE 71H2

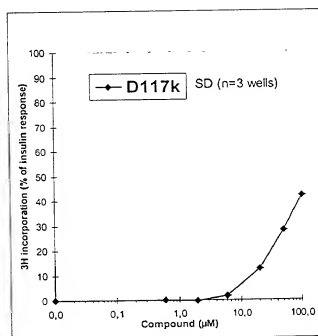


FIGURE 71I2

FIGURE 71J2

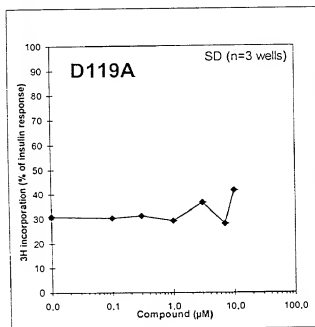
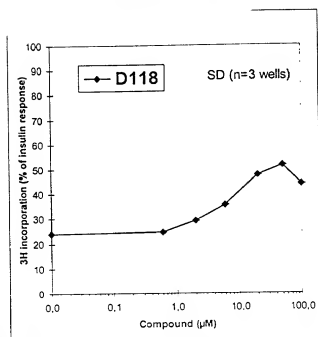


FIGURE 71K2

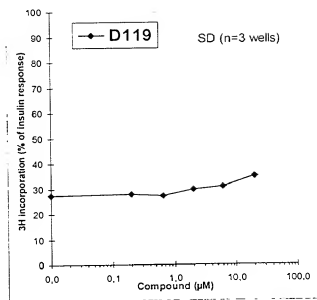


FIGURE 71L2

FIGURE 71M2

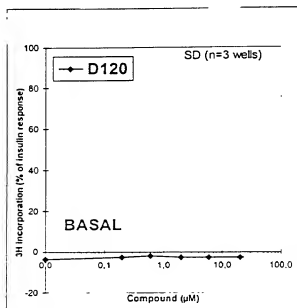


FIGURE 71N2

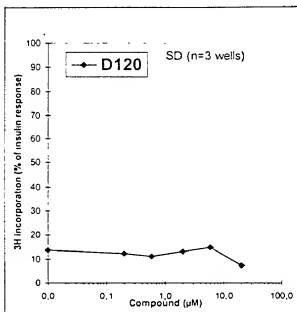
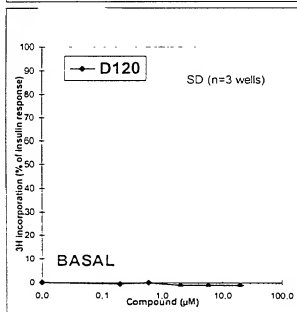
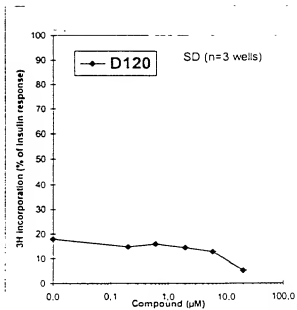


FIGURE 71O2

FIGURE 71P2

FIGURE 71Q2

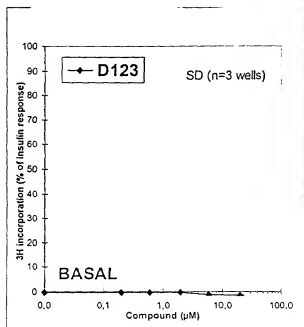


FIGURE 71R2

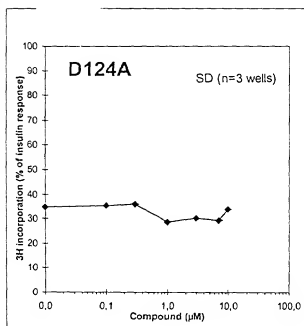
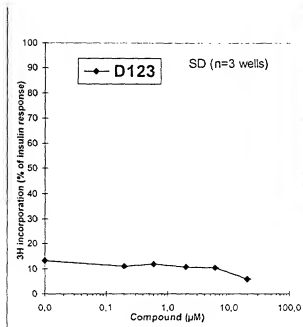


FIGURE 71S2

FIGURE 71T2

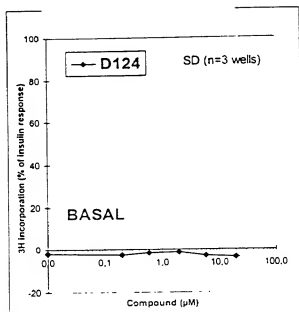


FIGURE 71U2

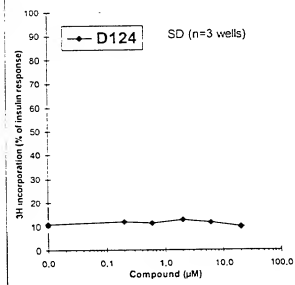
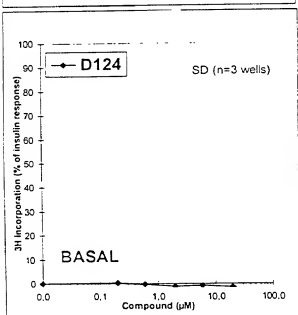
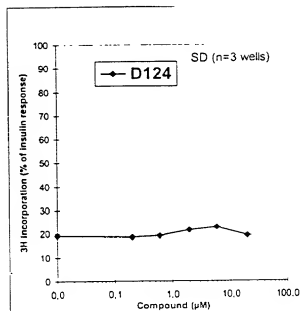


FIGURE 71V2

FIGURE 71W2

FIGURE 71Y2

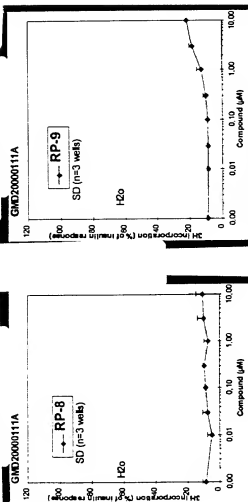


FIGURE 71Z2

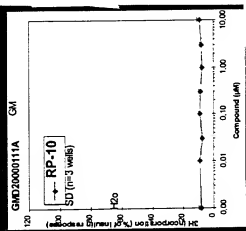


FIGURE 71A3

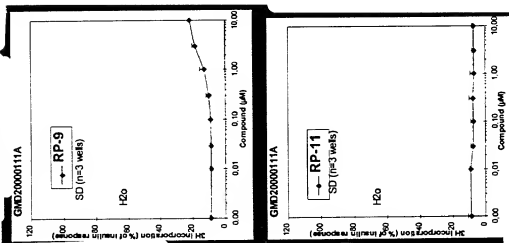


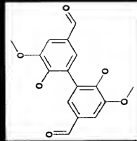
FIGURE 71Z3

FIGURE 71A4

S291: Dimer of S204 with linker 9

S204 = Lig-GGGFHENFYDWFVRQVSKK

Linker 9 =



HIR binding = 1.2×10^{-6}

FFC:

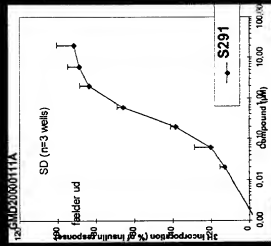


FIGURE 71B3

FIGURE 72A

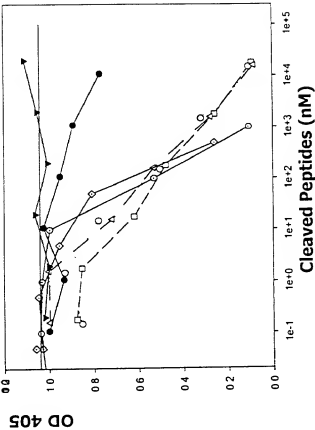
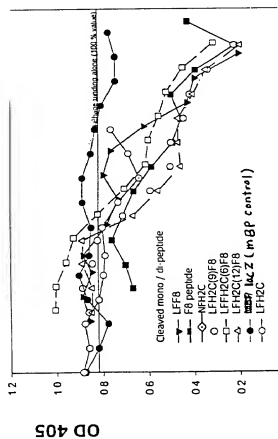


FIGURE 72B

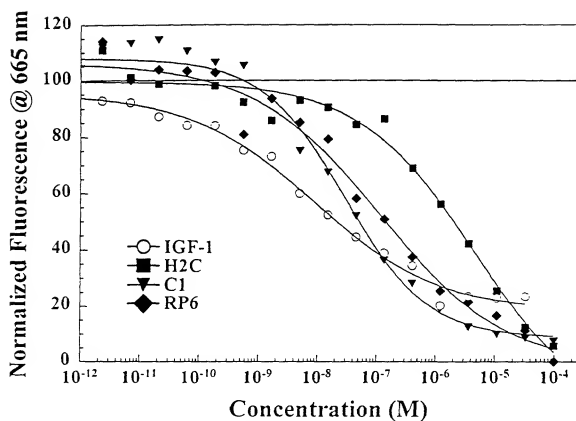


FIGURE 73

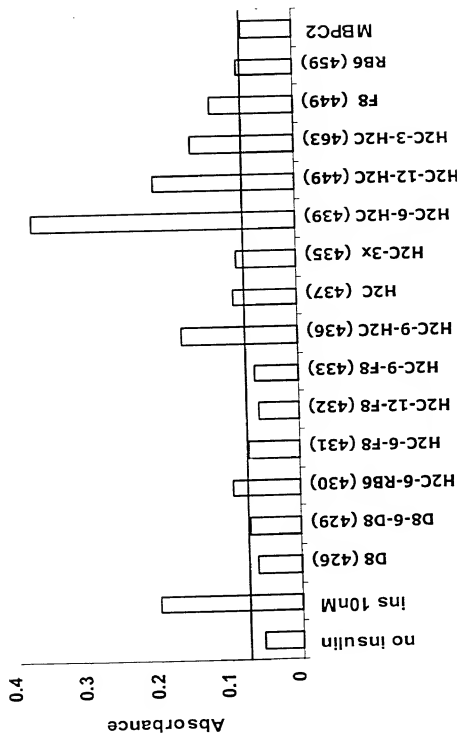


FIGURE 74

**COMBINED DECLARATION AND POWER OF ATTORNEY FOR
ORIGINAL, DESIGN, NATIONAL STAGE OF PCT, SUPPLEMENTAL
DIVISIONAL, CONTINUATION OR CONTINUATION-IN-PART APPLICATION**

As a below name inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

INSULIN AND IGF-1 RECEPTOR AGONISTS AND ANTAGONISTS

the specification of which

a. ☒ is attached hereto

b. ☐ was filed on _____ as application Serial No. _____ and was amended on _____ (if applicable).

PCT FILED APPLICATION ENTERING NATIONAL STAGE

c. ☐ was described and claimed in International Application No. _____ filed on _____ and as amended on _____ (if any).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

I hereby specify the following as the correspondence address to which all communications about this application are to be directed:

SEND CORRESPONDENCE TO: MORGAN & FINNEGAN, L.L.P.
345 Park Avenue
New York, N.Y. 10154

DIRECT TELEPHONE CALLS TO: (212) 758-4800

☐ I hereby claim foreign priority benefits under Title 35, United States Code § 119(a)-(d) or under § 365(b) of any foreign application(s) for patent or inventor's certificate or under § 365(a) of any PCT international application(s) designating at least one country other than the U.S. listed below and also have identified below such foreign application(s) for patent or inventor's certificate or such PCT international application(s) filed by me on the same subject matter having a filing date within twelve (12) months before that of the application on which priority is claimed:

☐ The attached 35 U.S.C. § 119 claim for priority for the application(s) listed below forms a part of this declaration.

<u>Country/PCT</u>	<u>Application Number</u>	<u>Date of filing (day, month, yr)</u>	<u>Date of issue (day, month, yr)</u>	<u>Priority Claimed</u>

[] YES [] NO

[] I hereby claim the benefit under 35 U.S.C. § 119(e) of any U.S. provisional application(s) listed below.

Provisional Application No.

Date of Filing (day, month, yr)

ADDITIONAL STATEMENTS FOR DIVISIONAL, CONTINUATION OR CONTINUATION-IN-PART
OR PCT INTERNATIONAL APPLICATION(S) (DESIGNATING THE U.S.)

I hereby claim the benefit under Title 35, United States Code § 120 of any United States application(s) or under § 365(c) of any PCT international application(s) designating the U.S. listed below.

U.S. Serial No. 09/146,127	September 2, 1998	Pending
US/PCT Application Serial No.	Filing Date	Status (patented, pending, abandoned)/ U.S. application no. assigned (For PCT)

[X] In this continuation-in-part application, insofar as the subject matter of any of the claims of this application is not disclosed in the above listed prior United States or PCT international application(s) in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or Imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint the following attorneys and/or agents with full power of substitution and revocation, to prosecute this application, to receive the patent, and to transact all business in the Patent and Trademark Office connected therewith: John A. Diaz (Reg. No. 19,550), John C. Vassil (Reg. No. 19,098), Alfred P. Ewert (Reg. No. 19,887), David H. Pfeffer (Reg. No. 19,825), Harry C. Marcus (Reg. No. 22,390), Robert E. Paulson (Reg. No. 21,046), Stephen R. Smith (Reg. No. 22,615), Kurt E. Richter (Reg. No. 24,052), J. Robert Dailey (Reg. No. 27,434), Eugene Moroz (Reg. No. 25,237), John F. Sweeney (Reg. No. 27,471), Arnold I. Rady (Reg. No. 26,601), Christopher A. Hughes (Reg. No. 26,914), William S. Feiler (Reg. No. 26,728), Joseph A. Calvaruso (Reg. No. 28,287), James W. Gould (Reg. No. 28,859), Richard C. Komson (Reg. No. 27,913), Israel Blum (Reg. No. 26,710), Bartholomew Verdirame (Reg. No. 28,483), Maria C.H. Lin (Reg. No. 29,323), Joseph A. DeGirolamo (Reg. No. 28,595), Michael P. Dougherty (Reg. No. 32,730), Seth J. Atlas (Reg. No. 32,454), Andrew M. Riddles (Reg. No. 31,657), Bruce D. DeRenzi (Reg. No. 33,676), Michael M. Murray (Reg. No. 32,537), Mark J. Abate (Reg. No. 32,527), Alfred L. Haffner, Jr. (Reg. No. 18,919), Harold Haidt (Reg. No. 17,509), John T. Gallagher (Reg. No. 35,516), Steven F. Meyer (Reg. No. 35,613), Kenneth H. Sonnenfeld (Reg. No. 33,285), Tony V. Pezzano (Reg. No. 38,271), Andrea L. Wayda (Reg. No. 43,979) and Walter G. Hanchuk Reg. No. (35,179) of Morgan & Finnegan, L.L.P. whose address is: 345 Park Avenue, New York, New York, 10154; and Michael S. Marcus (Reg. No. 31,727) and John E. Hoel (Reg. No. 26,279) of Morgan & Finnegan, L.L.P., whose address is 1775 Eye Street, Suite 400, Washington, D.C. 20006.

[] I hereby authorize the U.S. attorneys and/or agents named hereinabove to accept and follow instructions from

_____ as to any action to be taken in the U.S. Patent and Trademark Office regarding this application without direct communication between the U.S. attorneys and/or agents and me. In the event of a change in the person(s) from whom instructions may be taken I will so notify the U.S. attorneys and/or agents hereinabove.

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Full name of thirteenth joint inventor Ku-chuan Hsiao

Inventor's signature* _____

date _____

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[] ATTACHED IS/ARE ADDED PAGE(S) TO COMBINED DECLARATION AND POWER OF ATTORNEY FORM FOR SIGNATURE BY FOURTH AND SUBSEQUENT INVENTORS

* Before signing this declaration, each person signing must:

1. Review the declaration and verify the correctness of all information therein; and
2. Review the specification and the claims, including any amendments made to the claims.

After the declaration is signed, the specification and claims are not to be altered.

To the inventor(s):

The following are cited in or pertinent to the declaration attached to the accompanying application:

Title 37, Code of Federal Regulation, § 1.56

Duty to disclose information material to patentability.

(a) A patent by its very nature is affect with a public interest. The public interest is best served, and the most effective patent examination occurs when, at the time an application is being examined, the Office is aware of and evaluates the teachings of all information material to patentability. Each individual associated with the filing and prosecution of a patent application has a duty of candor and good faith in dealing with the Office, which includes a duty to disclose to the Office all information known to that individual to be material to patentability as defined in this section. The duty to disclose information exists with respect to each pending claim until the claim is canceled or withdrawn from consideration, or the application becomes abandoned. Information material to the patentability of a claim that is canceled or withdrawn from consideration need not be submitted if the information is not material to the patentability of any claim remaining under consideration in the application. There is no duty to submit information which is not material to the patentability of any existing claim. The duty to disclose all information known to be material to patentability is deemed to be satisfied if all information known to be material to patentability of any claim issued in patent was cited by the Office or submitted to the Office in the manner prescribed by §§1.97(b)-(d) and 1.98. However, no patent will be granted on an application in connection with which fraud on the Office was practiced or attempted or the duty of disclosure was violated through bad faith or intentional misconduct. The Office encourages applicants to carefully examine:

- (1) prior art cited in search reports of a foreign patent office in a counterpart application,
- and

- (2) the closest information over which individuals associated with the filing or prosecution of a patent application believe any pending claim patentably defines, to make sure that any material information contained therein is disclosed to the Office.

Title 35, U.S. Code § 101

Inventions patentable

Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

Title 35 U.S. Code § 102

Conditions for patentability; novelty and loss of right to patent

A person shall be entitled to a patent unless —

- (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for patent,
- (b) the invention was patented or described in a printed publication in this or foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States, or
- (c) he has abandoned the invention, or
- (d) the invention was first patented or caused to be patented, or was the subject of an inventor's certificate, by the applicant or his legal representatives or assigns in a foreign country prior to the date of the application for patent in this country on an application for patent or inventor's certificate filed more than twelve months before the filing of the application in the United States, or
- (e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent, or
- (f) he did not himself invent the subject matter sought to be patented, or
- (g) before the applicant's invention thereof the invention was made in this country by another had not abandoned, suppressed, or concealed it. In determining priority of invention there shall be considered not only the respective dates of conception and reduction to practice of the invention, but also the reasonable diligence of one who was first to conceive and last to reduce to practice, from a time prior to conception by the other ...

Title 35, U.S. Code § 103

Conditions for patentability; non-obvious subject matter

A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said matter pertains. Patentability shall not be negated by the manner in which the invention was made.

Subject matter developed by another person, which qualifies as prior art only under subsection (f) or (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

Title 35, U.S. Code § 112 (in part)

Specification

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise and exact terms also enable any person skilled in the art to which it pertains, or with which it is mostly nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Title 35, U.S. Code § 119

Benefit of earlier filing date in foreign country; right of priority

An application for patent for an invention filed in this country by any person who has, or whose legal representatives or assigns have, previously regularly filed an application for a patent for the same invention in a foreign country which affords similar privileges in the case of applications filed in the United States or to citizens of the United States, shall have the same effect as the same application would have if filed in this country on the date on which the application for patent for the same invention was first filed in such foreign country, if the application in this country is filed within twelve months from the earliest date on which such foreign application was filed; but no patent shall be granted on any application for patent for an invention which had been patented or described in a printed publication in any country more than one year before the date of the actual filing of the application in this country, or which had been in public use or on sale in this country more than one year prior to such filing.

Title 35, U.S. Code § 120

Benefit or earlier filing date in the United States

An application for patent for an invention disclosed in the manner provided by the first paragraph of section 112 of this title in an application previously filed in the United States, or as provided by section 363 of this title, which is filed by an inventor or inventors named in the previously filed application shall have the same effect, as to such invention, as though filed on the date of the prior application, if filed before the patenting or abandonment of or termination of proceedings on the first application or an application similarly entitled to the benefit of the filing date of the first application and if it contains or is amended to contain a specific reference to the earlier filed application.

Please read carefully before signing the Declaration attached to the accompanying Application.

If you have any questions, please contact Morgan & Finnegan, L.L.P.

FORM:COMB-DEC.NY
Rev. 5/21/98